YOUR GOOD PARTNER IN BIOLOGY RESEARCH

Contact us:

Tel.: 915 515 403

Fax: 914 334 545

e-mail: info@bionova.es

www.bionova.es

CUSABIO
PROTEIN EXPRESSION SERVICE

More than **10** years' experience

11100+ orders completed

99.1% success rate

Cusabio Protein Expression Platform

Why choose us?

- **Risk-free: Do not charge by steps. No protein, No charge**
- Competitive price as low as **$535**
- 39 kinds of tags meeting different demands
- Secondary AKTA-SEC purification to ensure high purity
- Ability to achieve large-scale production (10 mg, 50 mg, 100 mg, 200 mg available)
- Post-purification services available: Desalting, aliquot, endotoxin removal, aseptic process and lyophilization

Platform advantage

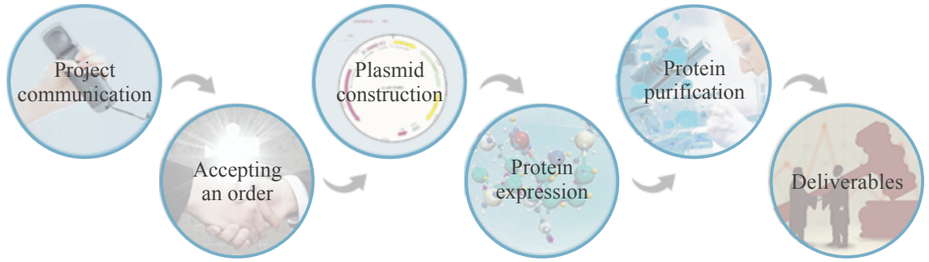
The characteristics of proteins expressed by different expression systems

Characteristic		E.coli	Yeast	Insect	Mammalian	Cell Free
Yield		high	high	medium	low	medium
Speed		fast	medium	slow	slow	fast
Cost		low	low	medium	high	high
Secretion		to periplasm	to medium	no	to medium	no
Growth medium		simple	simple	complex	complex	complex
Folding		poor	efficient	efficient	efficient	poor
Glycosylation	N-linked	no	high mannose	complex	complex	no
	O-linked	no	yes	yes	yes	no
Phosphorylation		no	yes	yes	yes	no
Acetylation		no	yes	yes	yes	no
Large MW protein expression		NR	NR	yes	NR	NR
Transmembrane/Toxic protein expression		NR	NR	NR	NR	yes
Scale up		****	****	**	*	***

Note: NR means not recommended.

bioNova científica, s.l. · Tel.: 915 515 403 · Fax: 914 334 545 · e-mail: info@bionova.es · www.bionova.es

Service process



Required

Target protein information or gene sequences.

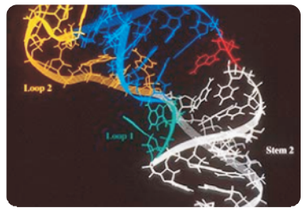
Final Deliverables

Purified protein, the purity is more than 85%;
Standard COA report as well as datasheet, including tag information, molecule weight, electrophoretic parameters, protein expression quantity, concentration, purity, SDS-PAGE, etc.

Which expression system suits your experiment most ?

Expression System	System Benefits	Application	Features of Cusabio
In vitro E.coli Expression System	Simple, take short time, high expression quantity, open and flexible, easy to express specific proteins, prepare protein complexes, parallel to synthesize a variety of different proteins, etc.	Toxic proteins, membrane proteins	Small amount expression conditions fumble, solve relative problems professionally, greatly reducing the experimental period, increase the expression quantity
E. coli Expression System	High target gene expression quantity, low cost, simple culture conditions, product rapidly, strong scalability, simple conversion operation, easy to form disulfide bond	Prokaryotic proteins, simple eukaryotic proteins	Expression includes soluble protein, inclusion body, fusion proteins, etc., with wealthy experience and expertise, we can solve a variety of bottlenecks during the protein expression process
Yeast Expression System	Cost-effective, low-cost for amplifying medium, simple culture conditions, production rapidly, strong scalability, good choice for secretory protein or intracellular protein expression, secrete proteins efficiently and allow simple purification, extensive post-translational modifications, no endotoxin	Industrial strain improvement, amplification	The combination of self-transformed efficient secretion vector and host can achieve the highest quality protein expression to the maximum extent; Patented Biobrick technology can be successfully used to the improvement and optimization of industrial strain
Insect Baculovirus Expression System	Large gene capacity, high efficiency of exogenous gene expression, effective cell fold, moderate scalability, extensive post-translational modifications, glycosylation similar to mammalian cells, is relatively easy enzymatic deglycosylation, no endotoxin	Virus vaccines, signal proteins, cytokines, kinases, etc.	Adopt AcNPV-sf9 cells and high5 cells two expression systems, the selectivity of multiple expression systems, multiple hosts, multi-carrier greatly improve the success rate of protein expression
Mammalian Cell Expression System	Higher expression levels, moderate scalability, cell suspension culture characteristic can do mass production, effective protein fold, suitable for protein secretion, full post-translational modifications, no endotoxin	Complex higher eukaryotes proteins	Adopt the specific combined methods of mammalian cell expression vector and a variety of transfection, optimize expression conditions, improve transfection efficiency, greatly shorten the experimental period, significantly increase the expression quantity

In vitro E.coli Expression System



The cell-free protein expression system is also known as the *in vitro* translation system. The cell-free protein synthesis system uses the target mRNA or DNA as the template, adds the substrate and energy required for the protein synthesis to the enzyme system from the cell extract, and synthesize the target protein *in vitro*. The cell-free protein expression system simulates *in vivo* cells and reproduces the intracellular protein transcription and translation process. It needs the existence of various materials required for protein synthesis, including energy, transcription factors, and translation factors, etc.

The system is particularly suitable for the expression of transmembrane proteins and toxic proteins. Its feature includes short cycle and high-throughput.

Even though the system has more than 10 years of history, it still has some technical difficulties. Currently in the global base, cell-free protein expression is mainly provided through Rothe, Promega and other companies using kit expression, which has only very limited conditions and be very expensive. Our company is the first company to master the full set of core technology of E.coli cell-free expression system in domestic market, all core components are produced in house, and the reaction system contains more than 40 ingredients, which are easy to be adjusted and optimized. Since the establishment of this platform in 2015, 162 proteins have been successfully produced with yield of mg/ml, which contains 99 transmembrane proteins with 1-12 transmembrane domains and toxic proteins that are difficult to express in traditional E.coli expression systems. We have also produced high molecular weight proteins (130 kDa -140 kDa) that contain multiple transmembrane domains.

Advantages

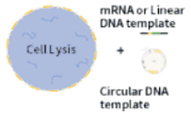
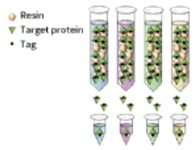
Compared with the traditional intracellular protein expression system, the cell-free system has the following significant advantages:

- **High yield**, some protein can reach as high as 5 mg/ml. Currently, most of membrane protein data are obtained from the E. coli cell-free expression system
- **No restrictions on cell structure**, it can express exogenous proteins that are toxic to the host cells
- **High-throughput**, it allows expression of several different proteins simultaneously on the multi-well plate under a variety of different conditions. It is suitable for high-throughput proteomics research
- **The open reaction system makes the reaction conditions easy to change**, which is helpful to regulate gene transcription, protein synthesis and post-translational modification
- **Allow addition of non-natural amino acids or isotope-labeled amino acids** to synthesize proteins for special use
- **Less steps**, simple experimental process, low dependence on equipment
- **Price as low as $825**, delivery time as short as 25 business days

Guarantee

Risk-free: We do NOT charge if we cannot deliver the protein.

Service Process

Steps	Project	Process		Cusabio Features	Lead Time
1	Plasmid construction and preparation 	Codon optimization, gene synthesis		**Multi-vector optimization** In order to improve the efficiency of mRNA translation, thereby increasing protein yield, we provide protein expression service using N-terminal peptide optimization in addition to conventional N-terminal fusion protein. The N-terminal peptide contains 6-11 amino acids, it's the shortest additional amino acid sequence that we have designed.	15-20 business days
		The PCR amplification products are ligated to the pET vectors by restriction enzyme digestion			
		Recombinant plasmids are prepared in large quantities			
2	Small-scale expression and optimization 	Multi-condition optimization; SDS-PAGE electrophoresis; Determine the optimal reaction condition		**Multi-condition expression scheme** In cell-free expression system, we can express several different proteins simultaneously on the multi-well plate under a variety of different conditions. Thus we offer multi-condition optimization service.	7-10 business days (Additional 3 business days for multi-condition purification)
3	Target protein expression and purification 	Prepare 1-10ml large-scale expression based on the small-scale results			
		The target protein is purified by exploring different chromatographic conditions including ion exchange chromatography, size exclusion chromatography and others by using AKTA, and then determine the optimal purification method.		**Multi-condition purification scheme (optional)** For transmembrane proteins, we provide different detergent purification services to determine the optimum buffer for your transmembrane protein. This purification scheme is most suitable for transmembrane proteins with bioactivity.	
4	Additional services (optional) 	Charge	Tag-removal service	**Flexible additional services** Customers can flexibly choose from a variety of additional services to their specific needs, e.g. Endotoxin removal, Filter-sterilization, Tag removal, Lyophilization, etc. Some are complimentary, and some require additional charge.	3 business days
		Free	Endotoxin removal, Filter-sterilization, Lyophilization (Note: Lyophilization and filter-sterilization can not be met at the same time)		2 business days
5	Quality Control 	Testing of purity, concentration, etc. QC report is provided.		**Detailed COA report** Detailed product data sheet and COA are provided for each project.	3-5 business days
Total lead time					25-35 business days

In vitro E.coli Expression System

Project showcase

◆Case 1

The following three items are proteins with 5, 6 and 7 transmembrane domains separately. Since the *in vivo* expression system is difficult to express multiple transmembrane proteins, or the yield is very low, CUSABIO use cell-free expression system to produce these three proteins. To increase the yield, we explored a variety of expression conditions for the customer. Figure 1, 2, and 3 have shown the small scale expression of these three proteins under different conditions, and we selected the optimal condition for the large-scale expression.

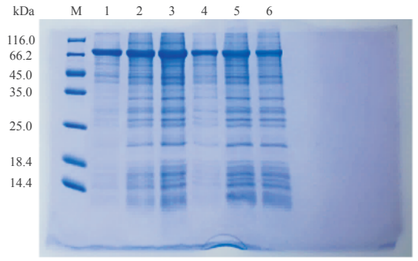


Figure 1. Ion Gradient Optimization of FiveTransmembrane Protein

Lane 1: Reaction Condition 1
Lane 2: Reaction Condition 2
Lane 3: Reaction Condition 3
Lane 4: Reaction Condition 4
Lane 5: Reaction Condition 5
Lane 6: Reaction Condition 6

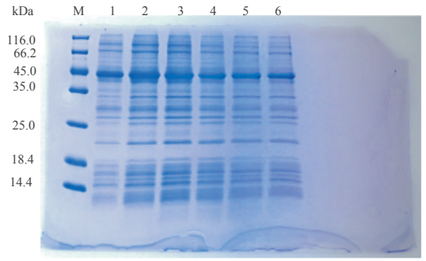


Figure 2. Ion Gradient Optimization of Six Transmembrane Protein

Lane 1: Reaction Condition 1
Lane 2: Reaction Condition 2
Lane 3: Reaction Condition 3
Lane 4: Reaction Condition 4
Lane 5: Reaction Condition 5
Lane 6: Reaction Condition 6

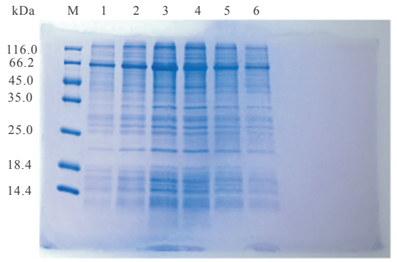


Figure 3. Ion Gradient Optimization of SevenTransmembrane Protein

Lane 1: Reaction Condition 1
Lane 2: Reaction Condition 2
Lane 3: Reaction Condition 3
Lane 4: Reaction Condition 4
Lane 5: Reaction Condition 5
Lane 6: Reaction Condition 6

◆Case 2

The project was a 9 transmembrane protein. The difficulty of this project was not only the large number of transmembrane domains, but also the high molecular weight (141.7 kDa). After multiple-condition optimization, we successfully produced the protein with high yield, which can be observed on SDS-PAGE.

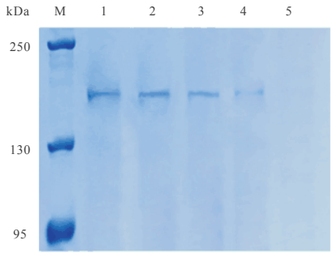
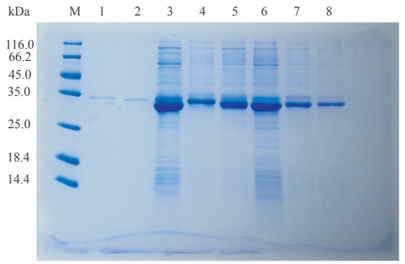


Figure 4. Ion Gradient Optimization of NineTransmembrane Protein

Lane 1: Reaction Condition 1
Lane 2: Reaction Condition 2
Lane 3: Reaction Condition 3
Lane 4: Reaction Condition 4
Lane 5: Reaction Condition 5

◆**Case 3**

The protein in this project had a very low yield. Through optimization of different N-terminal peptides, the yield was improved dramatically, as shown in Figure 5.

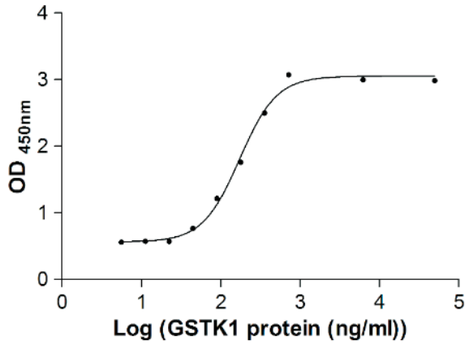


Lane 1: Tag 1
Lane 2: pET-28a(+) control
Lane 3: Tag 2
Lane 4: Tag 3
Lane 5: Tag 4
Lane 6: Tag 5
Lane 7: Tag 6
Lane 8: Tag 7

Figure 5. Different N-terminal Tags Optimization of Membrane Protein

◆**Case 4**

HTR1B is a membrane protein of the GPCR family. It contains 7 transmembrane domains. We successfully expressed this protein and did the functional activity test, and the result has shown that the protein is bioactive.

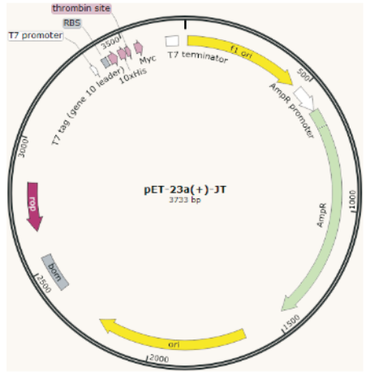


Activity: Measured by its binding ability in a functional ELISA. Immobilized HTR1B at 5 μg/ml can bind human GSTK1,the EC_{50} of human GSTK1 protein is 159.40-218.50 ng/ml.

Platform introduction

Characteristic expression systems

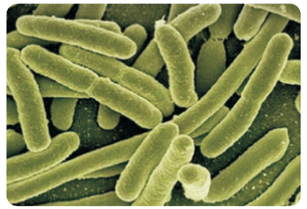
pET-23a(+)-JT vector, efficient expression *in vitro*



Vector characteristics:

1. Carrys T7 strong promoter, does not contain lac operon, no negative repressive effect, can express protein efficiently.
2. Compared with conventional 6xHis tag, the N-terminal 10xHis tag has a stronger binding ability in IMAC. The thrombin site makes it easy to remove the tag.
3. The C-terminal MYC-tag can be used for WB detection, and it has stronger sensitivity compared with His-tag.
4. Amp resistance screening.

E. coli Expression System



The E. coli expression system is regarded as the most commonly used, economical, and classical expression system because of its simple structure, clear genetic background, high yield of target protein, and its short culture period. In recent decades, E. coli expression system has also been developed and improved continuously, and been used intensively by scientific researchers and industrial users for a large number of recombinant protein expression. The system is mainly used for antigen preparation, ligand preparation, and expression of cytokines and bacteria (Staphylococcus aureus, Escherichia coli, etc.) proteins.

CUSABIO has extensive experience and be very professional in E. coli protein expression and purification. We can solve various difficult problems during the protein expression and purification process. From 2007 to 2017, we have successfully developed more than 4000 recombinant proteins expressed in E. coli, which contain hundreds of active proteins with high purity.

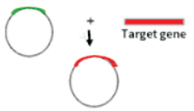
Advantages

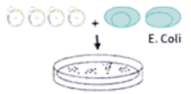
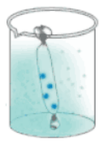
- Clear genetic background
- Cost-effective, easy for large-scale production. **Price as low as $535**
- **Short lead time**
- Fast growth and high yield: as high as 200 mg/L
- More options for vectors and tags, **higher success rate: as high as 99.1%**
- Easy to optimize various conditions to achieve the best results

Guarantee

Risk-free: We do NOT charge if we cannot deliver the protein.

Service Process

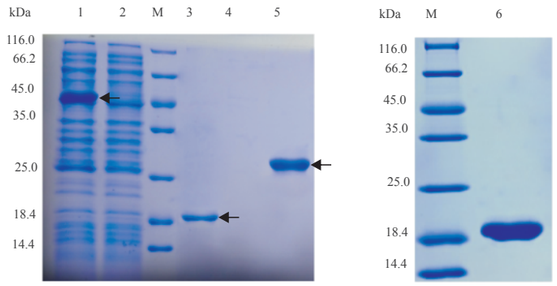
Steps	Project	Process	Cusabio Features	Lead Time
1	Plasmid construction 	Codon optimization; gene synthesis	**Multiple vectors optimization, More options for customers** Optimize multiple vectors at the same time; Select the vector that has the highest yield, which can shorten lead time;	15-20 business days
		Restriction digestion of PCR products; Ligation to expression vector, e.g. pCold-SUMO, pGEX-4T-1, pET22b-JT, etc.		
		Transform TOP10 E.coil competent cells		
		Obtain the correct recombinant plasmid		

2	Transformation and strain screening 	Transform the recombinant plasmid to host cells, e.g. BL21 (DE3), Rosetta-gami B (DE3) pLysS, C41 cells, culture overnight at 37℃	**Multi-conditions optimization, multi-hosts selection** In the small test, the temperature and IPTG are optimized to obtain the most suitable culture conditions. Multiple hosts are transformed at the same time to select the host bacteria with the highest yield.	5 business days
		Select single colony for small-scale induced expression; Detect protein expression by SDS-PAGE; Preserve the best colony.		
		Optimize the expression conditions		
3	Target protein expression and purification 	1-10 L large-scale expression	**Multiple purification methods (optional)** Explore different chromatographic conditions including ion exchange, hydrophobic and others by using AKTA, and then determine the optimal purification method.	12-15 business days
		Protein purification		
4	Inclusion body renaturation 	Refolding if the target protein is inclusion body	**Diverse refolding methods** A variety of buffering conditions are used to quickly screen the best refolding buffer formula. Refolding protein with purity greater than 90% is obtained by dilution renaturation, dialysis renaturation, column chromatography renaturation and so on. Solubilization and refolding can be achieved for more than 95% of inclusion bodies.	
5	Additional services (Optional) 	Charge	Tag removal by restriction digestion	**Flexible additional services** Customers can flexibly choose from a variety of additional services to their specific needs, e.g. Endotoxin removal, Filter-sterilization, Tag removal, Lyophilization, etc. Some are complimentary, and some require additional charge.	3 business days
		Free	Filter-sterilization; Endotoxin removal; Lyophilization (Note: Lyophilization and Filter-sterilization can not be met simultaneously)		2 business days
6	Quality Control 	Testing of purity, concentration, etc. QC report is provided.		**Detailed COA report** Detailed product data sheet and COA are provided for each project.	3-5 business days
Total lead time					35-45 business days

Project showcase

◆Case 1

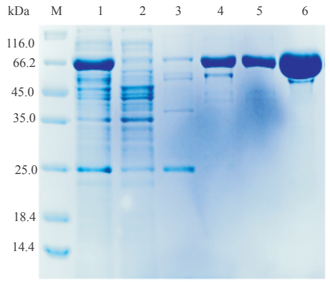
Characteristics: The protein is a fusion protein, and digested with PreScission protease overnight through the GST affinity chromatography column, subsequently with one-step purification to obtain protein without tag.



Lane 1 Cell lysate (Arrow indicates the target fusion protein)
Lane 2 Flow through
Lane 3 Protein digested by PreScission protease (Arrow indicates the target protein)
Lane 4 Remove impurity with PBS
Lane 5 GSH elution (Arrow indicates GST-tagged protein)
Lane 6 Concentrated target protein

◆Case 2

Characteristics: After expression, the target protein was purified by nickel column affinity chromatography. The purity reached 90%, and the yield reached 20 mg/L

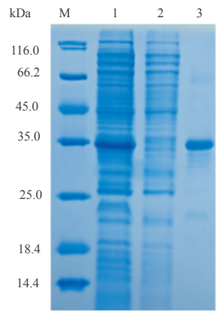


Lane 1 Cell lysate
Lane 2 Flow through
Lane 3 30 mM imidazole elution
Lane 4 60 mM imidazole elution
Lane 5 200 mM imidazole elution
Lane 6 500 mM imidazole elution

◆Case 3

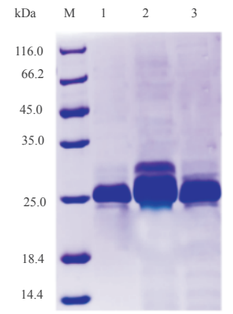
Characteristics: Multiple tag options can be provided for one protein.

▶ N-terminal GST-tagged



Lane 1 Cell lysate
Lane 2 Flow through
Lane 3 GSH elution

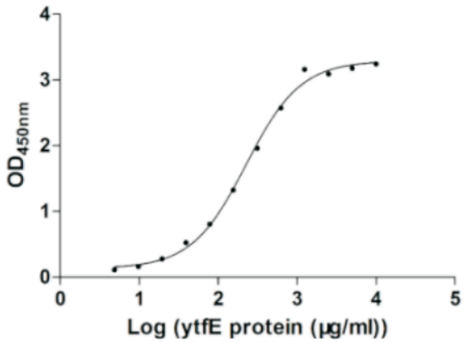
▶ N-terminal 6xHis-SUMO-tagged



Lane 1 60 mM imidazole elution and concentrate
Lane 2 200 mM imidazole elution and concentrate
Lane 3 500 mM imidazole elution and concentrate

◆**Case 4**

Characteristics: The recombinant protein is functional active.

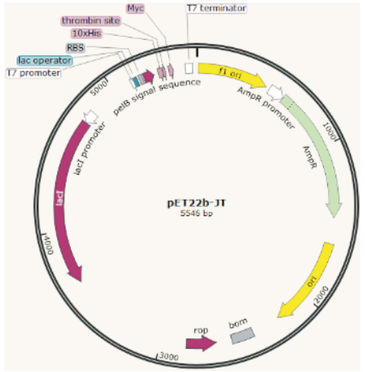


Activity: Measured by its binding ability in a functional ELISA. Immobilized aqpZ at 5 μg/ml can bind human ytfE, the EC_{50} of human ytfE protein is 197.90-259.70 μg/ml.

Platform introduction

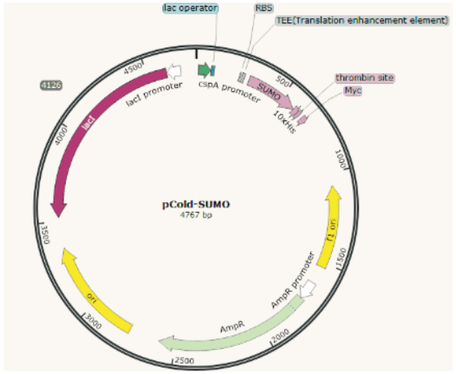
Characteristic expression systems

pET22b-JT plasmid + Rosetta-gami B (DE3) pLysS host bacteria Low temperature expression system



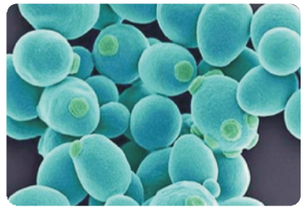
Vector characteristics:
1.Carry a T7 strong promoter; Contain PelB signal peptide; Low temperature induced secretory expression, which is conducive to correct protein folding and enhance protein solubility.
2.Seamless cloning, no restriction enzyme needed.
3.Compared with conventional 6xHis tag, the N-terminal 10xHis tag has a stronger binding ability in IMAC. Meanwhile the thrombin site makes it easy to remove the tag.
4.The C-terminal MYC-tag can be used for WB detection, and it has stronger sensitivity compared with His-tag.
5.Amp resistance screening.

pCold-SUMO plasmid + Rosetta-gami B(DE3)pLysS host bacteria low temperature expression system



Vector characteristics:
1.Carry cspA strong promoter; Contain SUMO fusion protein; possessing a strong ability to promote expression.
2.Seamless cloning, no restriction enzyme needed.
3.Compared with conventional 6xHis tag, the N-terminal 10xHis tag has a stronger binding ability in IMAC. Meanwhile the thrombin site makes it easy to remove the tag.
4.The C-terminal MYC-tag can be used for WB detection, and it has stronger sensitivity compared with His-tag.
5.Amp resistance screening.

Yeast Expression System



Yeast protein expression system is a highly economical eukaryotic expression system that do both secretion expression and intracellular expression. The exogenous gene expressed by Yeast expression system has a certain post-translational processing capacity, the expressed exogenous protein has a certain degree of folding and glycosylation modification, it's more stable than prokaryotic expressed proteins, particularly suitable for the expression of eukaryotic genes and preparation of functional proteins. Yeast secretion expression can secrete the expressed exogenous protein into the extracellular matrix, so that it is easy to obtain a high purity protein. Yeast expression system has many advantages which make its research and application more and more widely.

Advantages

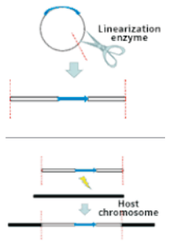
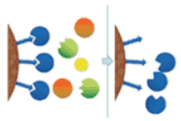
- **Cost-effective**, high expression level: as high as 100 mg/L (Shake flask culture)
- No self-produced endotoxin
- Products have **post-translational modifications**: glycosylation, phosphorylation, acylation, etc., it is more likely to have biological activity
- Products can be properly folded and efficient secretion
- It is more stable than prokaryotic proteins, and it is particularly suitable for the expression and preparation of functional proteins such as tuberculosis proteins, defensin, interleukin and cytokines
- **With patented Biobrick technology**, we can achieve efficient *in vitro* construction of any copy of the gene dose
- **Unique PickRight technology**, thus can directly obtain high expression level strains without screening after transformation, the time compared to traditional screening reduced 5-10 business days
- **Price as low as $780**, delivery time as short as 35 business days

Guarantee

Risk-free: We do NOT charge if we cannot deliver the protein.

Service Process

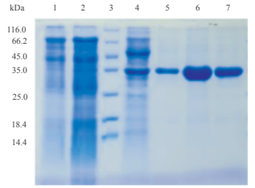
Steps	Project	Process	Cusabio Features	Lead Time
1	Expression vector construction 	Codon optimization; gene synthesis The PCR amplification products are ligated to the expression vectors e.g. pPic9k, pPic3.5k, pPiczαA, etc. Transform ligation mixtures into *E. coli* strain Obtain the correct recombinant plasmid	**Multiple vectors optimization, More options for customers** In order to improve the success rate of expression and achieve higher yield, in addition to conventional N-terminal fusion protein expression, we also provide protein with C-terminal fusion label, in greater degree to ensure the activity while ensuring the purity.	15-20 business days

2	Transformation and strain identification 	Prepare the recombinant plasmid in large quantities	**High copy screening** Conduct multiple screening through unique screening markers of different vectors, and the highest expression level strain was gradually obtained.	**PickRight Technology** The high expression level strain was obtained directly after transformation, and the time was shorten by 5-10 business days compared with the traditional screening. (Theoretically this technology is mainly recommended for the production of less than 5 mg/L protein expression)	10-13 business days
		Linearization of recombinant plasmid			
		Transform to GS115, X33, KM71 and other hosts by electroporation			
		PCR analysis is recommended to verify successful transformants			
		Use geneticin G418, Zeocion and other antibiotics for multiple copies screening to obtain high copy			
3	Small test, scale up expression and purification 	Small scale expression screening (20-40 strains)			7-12 business days/15-25 business days (Featured Purification)
		Determine strain and optimize expression conditions			
		Scale up culture			
		Protein purification		**Multiple purification methods (optional)** Explore different chromatographic conditions including ion exchange, hydrophobic and others by using AKTA, and then determine the optimal purification method.	
4	Additional services (Optional) 	Charge	Tag removal service	**Flexible additional services** Customers can flexibly choose from a variety of additional services to their specific needs, e.g. Endotoxin removal, Filter-sterilization, Tag removal, Lyophilization, etc. Some are complimentary, and some require additional charge.	3 business days
		Free	Filter-sterilization; Endotoxin removal; Lyophilization (Note: Lyophilization and filter-sterilization can not be met simultaneously)		2 business days
5	Quality Control 	Testing of purity, concentration, etc. QC report is provided.		**Detailed COA Report** Detailed product data sheet and COA are provided for each project.	3-5 business days
Total lead time					35-50 business days

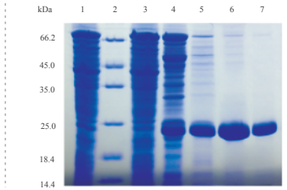
Project showcase

◆Case 1: High Purity Protein

Difficulty: Yeast intracellular expression, many impurities can be observed in the lysate protein (lane 1), the target protein was not obvious, after the yeast system-specific chromatography system purification, less miscellaneous band was observed with SDS-PAGE detection, the purity reached more than 95% (lane 6-7).

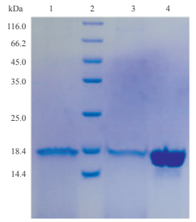


Lane 1 Lysate
Lane 2 Flow through
Lane 3 Marker
Lane 4-7 Target protein by different gradient elution



Lane 1 Lysate
Lane 2 Marker
Lane 3 Flow through
Lane 4-7 Target protein by different gradient elution

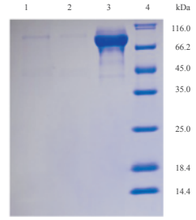
Features: Yeast secretion expression, the expressed protein was directly secreted into the medium, basically no impurities, the purity is as high as 90% or more, the purification is mainly for removing pigment and other residues in the medium, leaving only the target protein in the appropriate buffer.



Lane 1 Culture medium supernatant
Lane 2 Marker
Lane 3 Flow through
Lane 4 The eluted target protein

◆Case 2: Large Molecule Weight Protein Expression

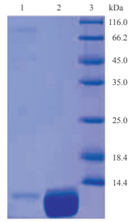
Difficulty: More than 700 amino acids, Mw: 81 kDa, we chose secretory vector for expression in order to get a higher purity, finally the protein was successful expressed and secreted into the culture medium.



Lane 1 Culture medium supernatant
Lane 2 Flow through
Lane 3 The eluted target protein
Lane 4 Marker

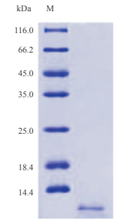
◆Case 3: Small Molecule Weight Protein Expression

Difficulty: 62 amino acids, Mw: 7 kDa, the molecular weight is very small, relatively difficult to concentrate and detect the protein. The expression, purification, collection were very successful from the SDS-PAGE detection result.



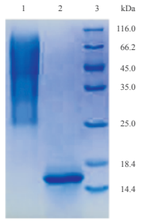
Lane 1 Culture medium supernatant
Lane 2 The eluted target protein
Lane 3 Marker

Difficulty: About 36 amino acids, Mw: 4 kDa, and this customer required to remove the tag, it's rather difficult to remove the tag as the molecular weight itself is very small, but we have successfully remove the tag after a series processing, and WB detection result showed the target protein didn't contain tag, so finally high-purity, low-molecular-weight and untagged protein was obtained.



◆Case 4: N-linked glycosylation modification (Yeast expression system unique characteristics)

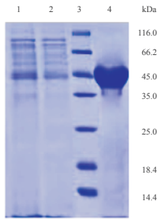
Features: Close to the modification of native proteins, especially glycosylation in the Yeast expression system is particularly evident by SDS-PAGE detection, it showed diffuse band and a large molecular weight, after digested by Endo H, the band was shaped and the size is consistent with the theoretical value.



Lane 1 Purified target protein (N-linked glycosylated modification)
Lane 2 Protein digested by Endo H enzyme
Lane 3 Marker

◆Case 5: pPic9k-SUMO Vector

Unique SUMO tag fusion protein

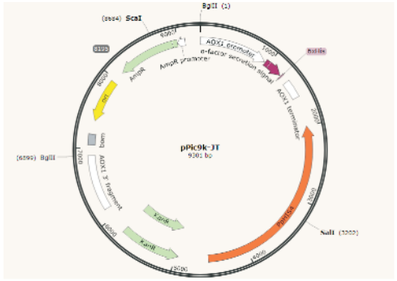


Lane 1 Culture medium supernatant
Lane 2 Flow through
Lane 3 Marker
Lane 4 SUMO tag fusion protein

Platform introduction

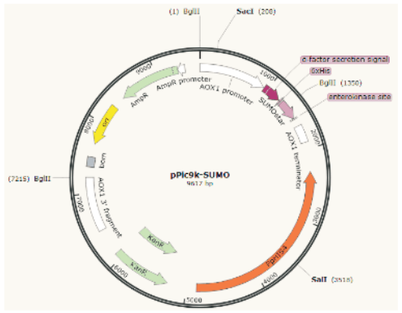
Characteristic expression systems

pPic9k-JT Plasmid+GS115 strain efficient expression



1.Carrys AOX1 strong promoter, containing alpha secreting factor which is able to do high efficient secretion expression.
2.Seamless cloning, no restriction enzyme needed.
3.Multiple linearization sites SalⅠ, SacⅠ, BglⅡ.
4.Can do Amp and Kan double-resistant screening to select positive strains during the prokaryotic stage.
5.Can do His+ and G418 screening to select high expression level strains during the eukaryotic stage.
6.Modified cloning sites, its cloning is not limited by the potential endonuclease in the target gene.
7.The vector has his tag, thus making it easy for cloning and purification.

pPic9k-SUMO Plasmid+GS115 strain efficient expression



1.Carry AOX1 strong promoter, containing alpha secreting factor which is able to do high efficient secretion expression.
2.Contain SUMO fusion protein; possessing a strong ability to promote expression.
3.Seamless cloning, no restriction enzyme needed.
4.Contain the EK cleavage site, can obtain untagged protein after enzyme digestion.
5.Multiple linearization sites SalⅠ, SacⅠ.
6.Can do Amp and Kan double-resistant screening to select positive strains during the prokaryotic stage.
7.Can do His+ and G418 screening to select high expression level strains during the eukaryotic stage.
8.Modified cloning sites, its cloning is not limited by the potential endonuclease in the target gene.
9.The vector has his tag, thus making it easy for cloning and purification.

Insect Baculovirus Expression System

Insect baculovirus expression vector system (BEVS) belongs to the eukaryotic expression system, and it's an expression system with high safety. It has a large genome, which enables the insertion of large exogenous genes, therefore has the great advantage of expressing proteins with large molecular weight. It also has the ability to achieve complete post-translational modification and efficiently express exogenous genes. The system consists of transfer vector, baculovirus vector and the host cell. The system uses one or more baculovirus super-strong promoters, and gets the recombinant virus after the exogenous target gene is inserted into the promoter. The highly efficient expression of the exogenous gene is achieved while the recombinant viruses replicate themselves in the insect cells. BEVS is widely used in virus vaccine development (such as the development of influenza virus vaccine and HPV vaccine), preparation of cell signaling proteins and cytokines, as well as kinase development, etc.

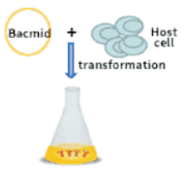
Advantages

- **Large capacity:** ability to carry large gene fragment; advantage in large protein expression
- **High safety:** baculovirus has strict species specificity
- **High expression efficiency:** the protein can be efficiently expressed in the late-stage infected cells
- The post-translational modification of the expressed product is similar to that of mammalian cells, particularly the glycosylation; the protein is more likely to be bioactive
- Baculovirus is easier to amplify and can produce recombinant proteins in **large scale**
- Our **unique bacmid based expression system** has prominent features including low cost, large volume suspension transfection, high titer, shorten lead-time by 1-2 weeks compare to classic process cycle
- **As low as $980**, delivery time as short as 7 weeks, yield up to 100 mg/L

Guarantee

Risk-free: We do NOT charge if we cannot deliver the protein.

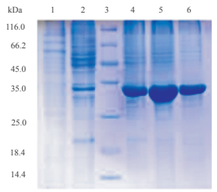
Service Process

Steps	Project	Process	Cusabio Features	Lead Time
1	Plasmid construction 	Codon optimization; gene synthesis The PCR product is ligated to the expression vectors e.g. pFastbac1-KHM, pFastBac1-MBP, etc. Transform TOP10 *E.coil* competent cells Obtain the correct recombinant plasmid	**Vector optimization** In order to improve the success rate of expression and achieve higher yield, we provide protein expression using C-terminal fusion tags in addition to conventional N-terminal tags, which retains the bioactivity of the protein while ensuring high purity.	15-20 business days
2	Preparation of recombinant Bacmid and high titer virus 	Transform DH10Bac cells to get recombinant Bacmid; PCR analysis; Isolate recombinant bacmid DNA Transfect recombinant Bacmid DNA into insect cells to obtain baculovirus, and detect expression level by SDS-PAGE; Repeat the infection if necessary	**Suspension transfection** Unique suspension transfection method greatly increases the protein expression level, and effectively shortens the experimental cycle.	12-15 business days
3	Scale up expression and purification	Infect insect cells with appropriate baculovirus The target protein is purified by affinity chromatography, ion exchange, hydrophobic and molecular sieves.	**Expression optimization** After optimization, the large amount of protein can be obtained by infecting host cells with low-passage virus.	5-10 business days
4	Additional services (optional) 	Charge: Tag removal by restriction digestion	**Flexible additional services** Customers can flexibly choose from a variety of additional services to their specific needs, e.g. Endotoxin removal, Filter-sterilization, Tag removal, Lyophilization, etc. Some are complimentary, and some require additional charge.	3 business days
		Free: Filter-sterilization; Endotoxin removal; Lyophilization (Note: Lyophilization and Filter-sterilization can't be met simultaneously)		2 business days
5	Quality Control 	Testing of purity, concentration, etc. QC report is provided.	**Detailed COA report** Detailed product data sheet and COA are provided for each project.	3-5 business days
Total lead time				35-50 business days

Project showcase

◆Case 1

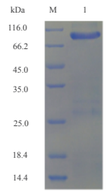
The protein was highly expressed in our company's Insect baculovirus expression vector system. A clear band was observed from cell lysate by SDS-PAGE. The yield was up to 20 mg/L after purification.



Protein purification image
Lane 1: Flow through.
Lane 2: Cell lysate
Lane 3: Marker
Lane 4: 30 mM imidazole elution
Lane 5: 60 mM imidazole elution
Lane 6: 250 mM imidazole elution

◆Case 2

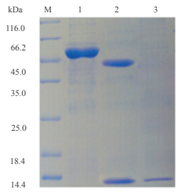
This target protein is relatively large. After gene synthesis, vector construction, bacmid construction, we finally produced the protein in sf9 cells. The yield reached to 5 mg/L, and the purity was 95%.



Protein purification image
Lane 1:60 mM imidazole elution

◆Case 3

The molecular weight of this target protein is relatively small, and it is quite difficult to express. We chose to use the pFastBac1-MBP vector to make the recombinant construct. After enzyme digestion and secondary purification, the purity of target protein reached 95%.

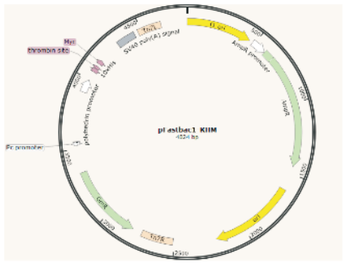


Protein purification image
Lane 1: Fusion protein before digestion
Lane 2: Fusion protein after digestion
Lane 3: Fusion protein after secondary purification

Platform introduction

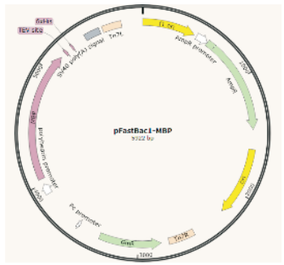
Characteristic expression systems

pFastbac1-KHM vector + sf9 cells, highly efficient expression system



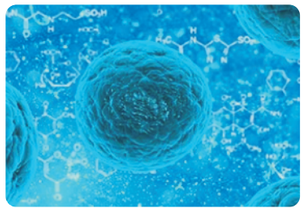
1.Seamless cloning, no restriction enzyme needed.
2.Compared with conventional 6xHis tag, the N-terminal 10xHis tag has a stronger binding ability in IMAC. The thrombin site makes it easy to remove the tag.
3.The C-terminal MYC-tag can be used for WB detection, and it has stronger sensitivity compared to His-tag.
4.Amp resistance screening.

pFastBac1-MBP vector+sf9 cell, highly efficient expression system



1.This vector contains MBP fusion tag that has a strong ability to promote expression.
2.It is a better system for small protein expression.
3.Seamless cloning, no restriction enzyme needed.
4.The TEV cleavage site makes it easy for tag removal.
5.Amp resistance screening.

Mammalian Cell Expression System



The prokaryotic expression system has the advantages of high expression level, simple operation, short cycle, easy large-scale and high-density culture and low cost. For the full-length antibody and glycoprotein biological drug, the folding of expression product polypeptide chain, the disulfide bond, the presence or absence of glycosylation and the type of glycosylation often affect the properties of the synthesis, secretion, biological activity, *in vivo* stability, and immunogenicity of the expressed product. Compared with other eukaryotic expression systems, the expression of the target gene in mammalian cells is similar to that of the native protein in the type and manner of the glycosylation, and can be correctly assembled into the multi-subunit protein.

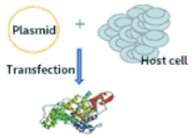
Advantages

- No self-produced endotoxin
- Secretion expression is available
- With a variety of complex **N-linked glycosylation, accurate O-linked glycosylation and other post-translational processing**
- **Close to native protein in the molecular structure**, physical and chemical properties and biological functions
- **High yield** after system optimization: as high as 100 mg/L
- Multi-cell lines, multi-expression methods to improve the protein expression success rate and protein yield
- **Price as low as $1298**, delivery time as short as 35 business days

Guarantee

Risk-free: We do NOT charge if we cannot deliver the protein.

Service Process

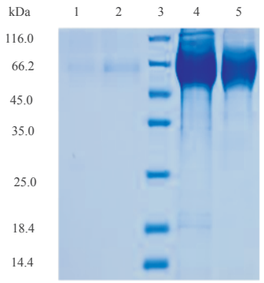
Steps	Project	Process		Cusabio Features	Lead Time
1	Plasmid construction 	Codon optimization; gene synthesis		**Multiple vectors optimization, More options for customers** In order to improve the success rate of expression and achieve higher yield, in addition to conventional N-terminal fusion protein expression, we also provide C-terminal fusion protein, which retains the bioactivity of the protein while ensuring high purity.	15-20 business days
		The PCR amplification products are ligated to the vectors e.g. pSec series, pCMV series and pcDNA series vectors			
		Transform TOP10 *E.coil* competent cells			
		Obtain the correct recombinant plasmid			
2	Small scale expression 	Prepare the transfection grade recombinant plasmid in large quantities		**Optimization of transfection conditions** Set different transfection conditions, select the optimal experimental conditions according to the test results.	9-11 business days
		Transient transfect HEK293, CHO and other cells			
		Detect expression products			
3	Scale up expression and purification 	Scale up the culture cells and transfect		**Multi-condition expression scheme** According to the protein localization and the best experimental conditions in the small test expression, select different cell lines and different ways of transfection, which can increase the expression quantity, greatly improve the protein expression.	8-9 business days
		Explore different chromatographic conditions including ion exchange, hydrophobic and others by using AKTA, and then determine the optimal purification method.			
4	Additional services (Optional)	Charge	Tag removal by restriction digestion	**Flexible additional services** Customers can flexibly choose from a variety of additional services to their specific needs, e.g. Endotoxin removal, Filter-sterilization, Tag removal, Lyophilization, etc. Some are complimentary, and some require additional charge.	3 business days
		Free	Filter-sterilization; Endotoxin removal; Lyophilization (Note: Lyophilization and Filter-sterilization can not be met simultaneously)		2 business days
5	Quality Control	Testing of purity, concentration, etc. QC report is provided.		**Detailed COA report** Detailed product data sheet and COA are provided for each project.	3-5 business days
Total lead time					35-45 business days

Mammalian Cell Expression System

Project showcase

◆Case 1

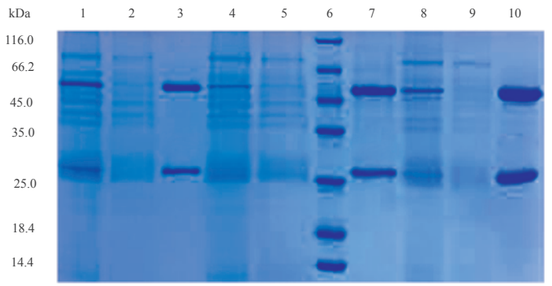
It is well known that the expression yield of mammalian cells is relatively low, the protein was optimally expressed with our mammalian expression system, the target band can be observed by SDS-PAGE analysis of the culture supernatants. The purified protein expression level can up to 10 mg/L. The theoretical molecular weight of the protein was 42 kDa, and the protein is modified with glycosylation by SDS-PAGE, which was confirmed by the examination of LC-MS/MS.



Lane 1 Flow through
Lane 2 Culture medium stoste
Lane 3 Marker
Lane 4 20 mM imidazole elution
Lane 5 60 mM imidazole elution

◆Case 2

Three full-length antibodies were transfected into CHO cells using our vector, after SDS-PAGE detection, the bands were observed in the supernatant of the culture medium, the expressed level was up to 100 mg/L.

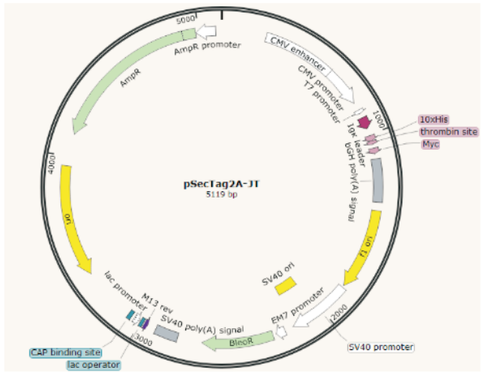


Lane 1 Antibody 1 culture medium stoste
Lane 2 Antibody 1 flow through
Lane 3 Antibody 1 elution
Lane 4 Antibody 2 culture medium stoste
Lane 6 Marker
Lane 7 Antibody 2 elution
Lane 8 Antibody 3 culture medium stoste
Lane 9 Antibody 3 flow through
Lane 10 Antibody 3 elution

Platform introduction

Characteristic expression systems

pSecTag2A-JT Vector+HEK293 Cell efficient expression



1.Carrys CMV strong promoter, containing IgK signal peptide which can enhance the secretory quantity of the protein.
2.Compared with conventional 6xHis tag, the N-terminal 10xHis tag has a stronger binding ability in IMAC. Meanwhile the thrombin site makes it easy to remove the tag to obtain untagged protein.
3.The C-terminal MYC-tag can be used for WB detection, and it has stronger sensitivity compared with His-tag.
4.Amp resistance screening.

Part of protein references are list as follows:

Title	Cat No	Product Name
Active surfaces engineered by immobilizing protein-polymer nanoreactors for selectively detecting sugar alcohols	CSB-EP360400ENV	Recombinant Enterobacter aerogenes Ribitol 2-dehydrogenase(rbtD)
Autophagy induced by DAMPs facilitates the inflammation response in lungs undergoing ischemia-reperfusion injury through promoting TRAF6 ubiquitination	CSB-EP010553PI	Recombinant Pig High mobility group protein B1(HMGB1)
Parallel optical read-out of micromechanical pillars applied to prostate specific membrane antigen detection	CSB-RP117294h	Recombinant Human Glutamate carboxypeptidase 2(FOLH),partial
Application of a SERS-based lateral flow immunoassay strip for the rapid and sensitive detection of staphylococcal enterotoxin B	CSB-YP360703FKZ	Recombinant Staphylococcus aureus Enterotoxin type B(entB)
Wentilactone A as a novel potential antitumor agent induces apoptosis and G2/M arrest of human lung carcinoma cells, and is mediated by HRas-GTP accumulation to excessively activate the Ras/Raf/ERK/p53-p21 pathway	CSB-EP010726HU	Recombinant Human GTPase HRas(HRAS)
Glial fibrillary acidic protein (GFAP) is a novel biomarker for the prediction of autoimmune diabetes	CSB-EP009369MO	Recombinant Mouse Glial fibrillary acidic protein(Gfap)
Schwann Cell Expressed Nogo-B Modulates Axonal Branching of Adult Sensory Neurons Through the Nogo-B Receptor NgBR	CSB-MP860384MO	Recombinant Mouse Nogo-B receptor(Nus1), partial
Association of chemical constituents and pollution sources of ambient fine particulate air pollution and biomarkers of oxidative stress associated with atherosclerosis: A panel study among young adults in Beijing, China	CSB-YP018840HU, CSB-E04675h	Recombinant Human Prostate stem cell antigen(PSCA)
Functional interaction between Lypd6 and nicotinic acetylcholine receptors	CSB-EP768237HU	Recombinant Human Ly6/PLAUR domain-containing protein 6(LYPD6)
Identification of three immunodominant motifs with atypical isotype profile scattered over the Onchocerca volvulus proteome	CSB-EP341251OEG	Recombinant Onchocerca volvulus OV-16 antigen(OV16),partial
Canstatin inhibits isoproterenol-induced apoptosis through preserving mitochondrial morphology in differentiated H9c2 cardiomyoblasts	CSB-YP005742MO	Recombinant Mouse Collagen alpha-2(IV) chain(Col4a2),partial
PDE5 Exists in Human Neurons and is a Viable Therapeutic Target for Neurologic Disease	CSB-YP847328DUB	Recombinant Clostridium acetobutylicum Asparagine--tRNA ligase(asnS)
Reactivity of anti-PEDV structural protein antibodies to porcine enteric coronaviruses: diagnostic implications	CSB-EP771125PPW	Recombinant Porcine epidemic diarrhea virus Envelope small membrane protein(E), Escherichia Coli
HMGB1/TLR4 signaling induces an inflammatory response following high-pressure renal pelvic perfusion in a porcine model	CSB-YP010553PI	Recombinant Pig High mobility group protein B1(HMGB1), Yeast
Identification and evaluation of potential forensic marker proteins in vaginal fluid by liquid chromatography/mass spectrometry	CSB-YP022619HU	Recombinant Human Small proline-rich protein 3(SPRR3)
Targeting of nucleoprotein to chemokine receptors by DNA vaccination results in increased CD8+-mediated cross protection against influenza	CSB-YP325878IMI	Recombinant Influenza A virus Nucleoprotein(NP)

bioNova científica, s.l. · Tel.: 915 515 403 · Fax: 914 334 545 · e-mail: info@bionova.es · www.bionova.es