

# Multi-pass Transmembrane Proteins and Technology Platforms

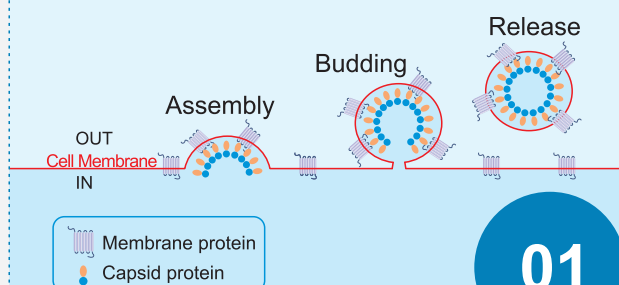
**FACILITATE DRUG DEVELOPMENT**

CUSABIO TECHNOLOGY LLC

Transmembrane protein (TP) is a protein that runs through both ends of biological membranes. TP can be divided into extracellular, transmembrane and intracellular domains. The interaction of the transmembrane region is an important channel connecting the intracellular and extracellular environments, and through this, various activation and response reactions are performed to achieve signal transduction, and to regulate the morphological and functional changes inside and outside the cell. Since TPs have hydrophobic and lipophilic transmembrane domains, the phospholipid bilayer of the cell membrane must be used to maintain the correct structure and maintain biological activity. The more transmembrane times, the more hydrophobic and lipophilic transmembrane regions, and the lower the expression level is in host cells. Therefore, it is a currently recognized technical difficulty to prepare active TPs *in vitro*.

Tps are currently the most important drug targets, accounting for more than 60% of the known drug targets. The R&D of innovative drugs targeting TPs has always been a research hotspot in the field of medicine. In order to help the development of transmembrane protein target drugs, CUSABIO has developed three powerful TPs technology platforms, including: virus-like particles (VLPs), detergents (Detergent Micelle) and Nanodisc platform. Currently, CUSABIO has successfully prepared more than 200 kinds of transmembrane proteins, including CCR8, C5AR1, Claudin-18.2 and other popular targets.

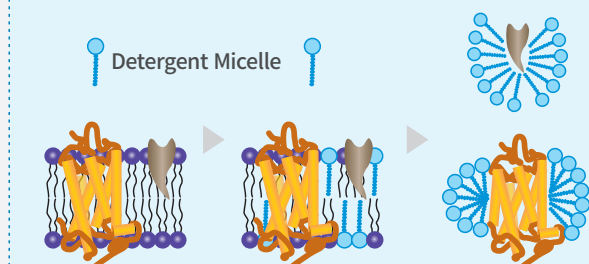
## Virus-like Particles (VLPs) Technology Platform



01

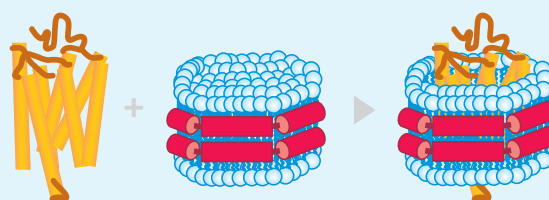
## Three Multi-pass Transmembrane Protein Technology Platforms

## Detergent Micelle Technology Platform



02

## 03 Nanodisc Technology Platform

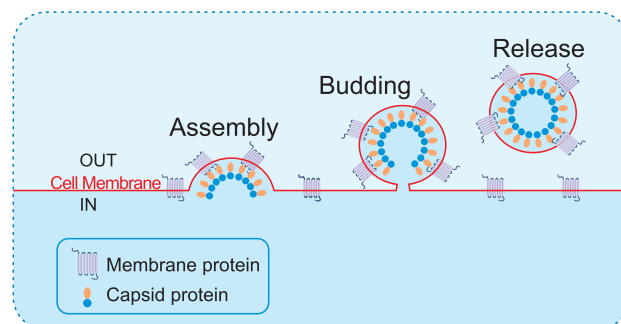


During the expression process of enveloped virus capsid protein, it can self-assemble into nanoparticles. After the expression of the target protein (transmembrane protein), it is located on the cell membrane, and VLP is released by budding. The transmembrane proteins are displayed on the envelope of the released nanoparticles (ie, VLPs particles).

In this platform, **CUSABIO mainly use the mammalian cell expression system for target protein expression**. The post-translational modification of the mammalian cell system is the closest to the native protein, and most pharmaceutical companies choose the drug target proteins from human species, so the Mammalian cell system is the best choice.

## Advantages

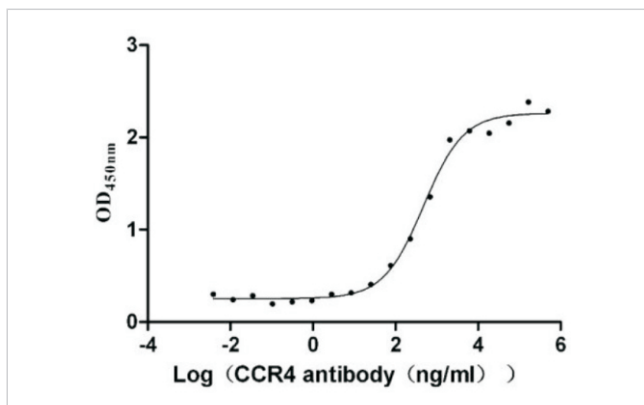
- Full-length TPs with complete natural conformation.
- Higher immunogenicity.
- Higher abundance than that of overexpressing cells.
- Can be the best targets for phage display because of their 100-200nm in size.
- Can be used in immunization/ELISA/SPR/BLI.



## Case Display

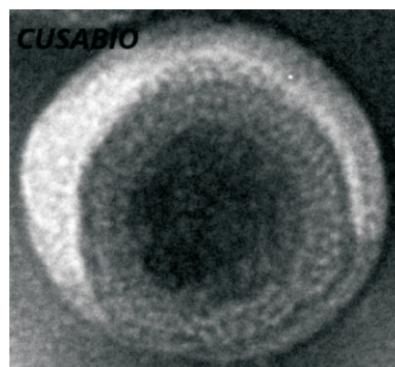
### CCR4-VLPs (7 times transmembrane) (CSB-MP004843HU)

#### Validated Data 1: Bio-activity Determined by Functional ELISA



Measured by its binding ability in a functional ELISA. Immobilized Human CCR4 at 10 µg/ml can bind Anti-CCR4 recombinant antibody(CSB-RA004843MA01HU), the EC<sub>50</sub> is 362.3-630.8 ng/mL.

#### Validated Data 2: Accuracy of structure determined by TEM



The presence of VLP-like structures was confirmed by TEM

## Other proteins developed by VLPs technology platform

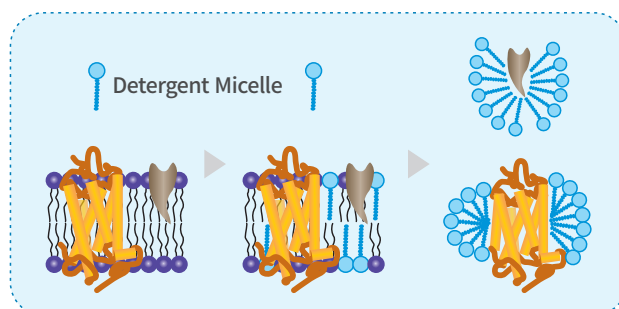
Target	Product Name	Transmembrane Times	Code
C5AR1	Recombinant Human C5a anaphylatoxin chemotactic receptor 1(C5AR1)	7	CSB-MP003996HU
CCR8	Recombinant Human C-C chemokine receptor type 8(CCR8)	7	CSB-MP004847HU
CD20	Recombinant Human B-lymphocyte antigen CD20(MS4A1)	4	CSB-MP015007HU
CLDN18.2	Recombinant Human Claudin-18.2(CLDN18.2)	4	CSB-MP005498HU(A5)
CXCR4	Recombinant Human C-X-C chemokine receptor type 4(CXCR4)	7	CSB-MP006254HU(F1)
GPRC5D	Recombinant Human G-protein coupled receptor family C group 5 member D(GPRC5D)	7	CSB-MP882153HU

Because of their strong hydrophobicity, transmembrane proteins are either difficult to dissolve in conventional solvents or cannot display the correct conformation. Appropriate detergents need to be added to aid solubility. CUSABIO has specially built a detergent technology platform based on cell free expression. Synthesize proteins by adding DNA templates, ATP, amino acids, various substrates, and enzymes derived from cell extracts in vitro, and then extract the target protein by screening different detergents.

In this platform, **CUSABIO uses in vitro E.coli expression system for the target protein expression**, then by screening different detergents to extract the target protein.

## Advantages

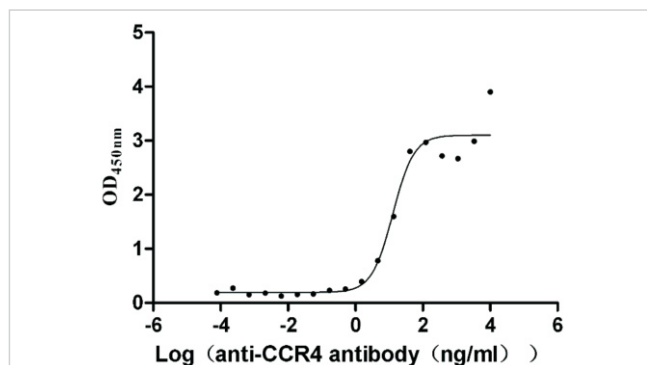
- Can express the full-length protein with multiple transmembrane domains, not limited to ECD epitopes.
- Precise quantification, distinct from VLPs and Nanodisc platforms.
- Can be applied to immunization/ELISA/SPR/BLI.



## Case Display

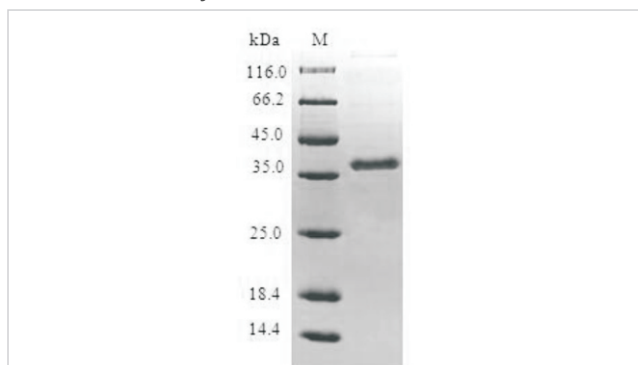
### CCR4-Detergent (7 times transmembrane) (CSB-CF004843HU)

#### Validated Data 1: Bio-activity Determined by Functional ELISA



Measured by its binding ability in a functional ELISA. Immobilized Human CCR4 at 5  $\mu\text{g/ml}$  can bind Anti-CCR4 recombinant antibody(CSB-RA004843MA01HU), the  $\text{EC}_{50}$  is 7.818-21.25 ng/mL.

#### Validated Data 2: Molecular Weight and Purity Determined by SDS-PAGE



Greater than 85% as determined by SDS-PAGE.

## Other Proteins developed by Detergent Micelle Technology Platform

Target	Product Name	Transmembrane Times	Code
AAP6	Recombinant Rice Amino acid permease(AAP6)	11	CSB-CF3147OCO
ABCD1	Recombinant Human ATP-binding cassette sub-family D member 1(ABCD1)	5	CSB-CF001068HU
ACKR1	Recombinant Human Atypical chemokine receptor 1(ACKR1)	7	CSB-CF624105HU
ACKR2	Recombinant Human Atypical chemokine receptor 2(ACKR2)	7	CSB-CF004618HU
ADIPOR1	Recombinant Human Adiponectin receptor protein 1(ADIPOR1)	7	CSB-CF001367HU
ADORA1	Recombinant Human Adenosine receptor A1(ADORA1)	7	CSB-CF001375HU
ADRB1	Recombinant Human Beta-1 adrenergic receptor(ADRB1)	7	CSB-CF001391HU(A4)
ADRB2	Recombinant Human Beta-2 adrenergic receptor(ADRB2)(G16R,E27Q)	7	CSB-CF001392HU(M)
ADTRP	Recombinant Human Androgen-dependent TFPI-regulating protein(ADTRP)	6	CSB-CF846640HUb1
ADTRP	Recombinant Human Androgen-dependent TFPI-regulating protein(ADTRP)	6	CSB-CF846640HUb3
AGTRAP	Recombinant Human Type-1 angiotensin II receptor-associated protein(AGTRAP)	3	CSB-CF744194HU
ALOX5AP	Recombinant Human Arachidonate 5-lipoxygenase-activating protein(ALOX5AP)	4	CSB-CF001625HU

Target	Product Name	Transmembrane Times	Code
APOLD1	Recombinant Human Apolipoprotein L domain-containing protein 1(APOLD1)	3	CSB-CF853458HU
AQP1	Recombinant Human Aquaporin-1(AQP1)	6	CSB-CF001957HU(A4)
AQPZ	Recombinant Escherichia coli Aquaporin Z (aqpZ)	6	CSB-CF352724ENV
B0W0I1	Recombinant Culex quinquefasciatus Odorant receptor(6031407)	4	CSB-CF3304DZM
btuC	Recombinant Halobacterium salinarum Cobalamin import system permease protein BtuC(btuC)	10	CSB-CF532244HTL(A4)
C5AR1	Recombinant Human C5a anaphylatoxin chemotactic receptor(C5AR1)	7	CSB-CF003996HUd7
C5AR2	Recombinant Human C5a anaphylatoxin chemotactic receptor 2(C5AR2)	7	CSB-CF868390HU
CACNA1C	Recombinant Guinea pig Voltage-dependent L-type calcium channel subunit alpha-1C(CACNA1C)	2	CSB-CF004399GU
CCR4	Recombinant Human C-C chemokine receptor type 4(CCR4)	7	CSB-CF004843HU
CCR5	Recombinant Human C-C chemokine receptor type 5(CCR5)	7	CSB-CF004844HU
CCR6	Recombinant Human C-C chemokine receptor type 6(CCR6)	7	CSB-CF004845HU
CCR6	Recombinant Human C-C chemokine receptor type 6(CCR6)	7	CSB-CF004845HUa2
Ccr8	Recombinant Mouse C-C chemokine receptor type 8(Ccr8)	7	CSB-CF004847MO
CCR8	Recombinant Human C-C chemokine receptor type 8(CCR8)	7	CSB-CF004847HU
CD37	Recombinant Human Leukocyte antigen CD37(CD37)	4	CSB-CF004928HU
CD37	Recombinant Bovine Leukocyte antigen CD37(CD37)	4	CSB-CF641596BO
Cd9	Recombinant Mouse CD9 antigen(Cd9)	4	CSB-CF004969MO
cea	Recombinant Escherichia coli Colicin-E1(cea)	2	CSB-CF360926ENL
CLCN3	Recombinant Human H(+)/Cl(-) exchange transporter 3(CLCN3)	15	CSB-CF005482HU(A4)
CLDN3	Recombinant Human Claudin-3(CLDN3)	4	CSB-CF005505HU
CNGA4	Recombinant Human Cyclic nucleotide-gated cation channel alpha-4(CNGA4)	6	CSB-CF808540HU
Cxcr2	Recombinant Mouse C-X-C chemokine receptor type 2(Cxcr2)	7	CSB-CF011673MO
CXCR3	Recombinant Human C-X-C chemokine receptor type 3(CXCR3)	7	CSB-CF006253HU
CXCR3	Recombinant Human C-X-C chemokine receptor type 3(CXCR3)	7	CSB-CF006253HUb0
CXCR4	Recombinant Human C-X-C chemokine receptor type 4(CXCR4)	7	CSB-CF006254HU
CXCR4	Recombinant Human C-X-C chemokine receptor type 4(CXCR4)	7	CSB-CF006254HUa0
CXCR7	Recombinant Human Atypical chemokine receptor 3(ACKR3)	7	CSB-CF006257HU(A4)
CYSLTR1	Recombinant Human Cysteinyl leukotriene receptor 1(CYSLTR1)	7	CSB-CF006465HU
EDNRA	Recombinant Human Endothelin-1 receptor(EDNRA)	7	CSB-CF007403HU
EDNRB	Recombinant Human Endothelin receptor type B(EDNRB)	7	CSB-CF007404HU
EMC4	Recombinant Human ER membrane protein complex subunit 4(EMC4)	2	CSB-CF023887HU
eptA	Recombinant Escherichia coli Phosphoethanolamine transferase eptA(eptA)	5	CSB-CF335584ENV
GCGR	Recombinant Human Glucagon receptor(GCGR)	7	CSB-CF009316HU
GLP1R	Recombinant Human Glucagon-like peptide 1 receptor(GLP1R)	7	CSB-CF009514HU(A4)
GNRHR2	Recombinant Macaca mulatta Gonadotropin-releasing hormone II receptor(GNRHR2)	7	CSB-CF856800MOW(M)
GPR146	Recombinant Human Probable G-protein coupled receptor 146(GPR146)	7	CSB-CF850271HU
Gpr146	Recombinant Mouse Probable G-protein coupled receptor 146(Gpr146)	7	CSB-CF857488MO
GPR157	Recombinant Human G-protein coupled receptor 157(GPR157)	7	CSB-CF713185HU
GPR35	Recombinant Human G-protein coupled receptor 35(GPR35)	4	CSB-CF881023HU
GPRC5D	Recombinant Human G-protein coupled receptor family C group 5 member D(GPRC5D)	7	CSB-CF882153HU
HTR1B	Recombinant Human 5-hydroxytryptamine receptor 1B(HTR1B)	7	CSB-CF010882HUb2
HTR1F	Recombinant Human 5-hydroxytryptamine receptor 1F(HTR1F)	7	CSB-CF010886HU
HTR2B	Recombinant Human 5-hydroxytryptamine receptor 2B(HTR2B)	7	CSB-CF010888HU
HTR7	Recombinant Human 5-hydroxytryptamine receptor 7(HTR7)	7	CSB-CF010899HU
Hvcn1	Recombinant Mouse Voltage-gated hydrogen channel 1(Hvcn1)	4	CSB-CF661026MO
IGSF1	Recombinant Human Immunoglobulin superfamily member 1(IGSF1)	2	CSB-CF854051HU(A4)
INSIG2	Recombinant Human Insulin-induced gene 2 protein(INSIG2)	6	CSB-CF896742HU
KCNJ10	Recombinant Human ATP-sensitive inward rectifier potassium channel 10(KCNJ10)	3	CSB-CF012048HU



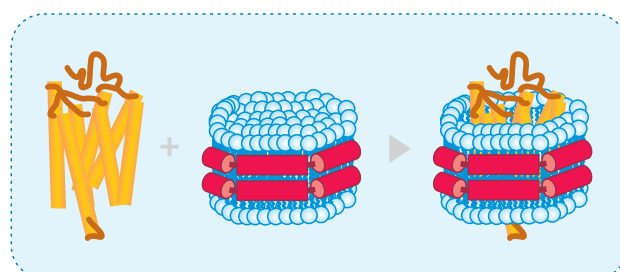
Target	Product Name	Transmembrane Times	Code
KCNK3	Recombinant Human Potassium channel subfamily K member 3(KCNK3)	4	CSB-CF012071HU
KCNN4	Recombinant Human Intermediate conductance calcium-activated potassium channel protein 4(KCNN4)	6	CSB-CF012086HU
lgt	Recombinant Lactococcus lactis subsp.lactis Prolipoprotein diacylglycerol transferase(lgt)	5	CSB-CF875009LNG
lspA	Recombinant Lactococcus lactis Lipoprotein signal peptidase(lspA)	3	CSB-CF2619Ba
M	Recombinant Human coronavirus NL63 Membrane protein(M)	3	CSB-CF757672HIX
MARCH2	Recombinant Human E3 ubiquitin-protein ligase MARCH2(MARCH2)	2	CSB-CF878948HU
mcr1	Recombinant Escherichia coli Probable phosphatidylethanolamine transferase Mcr-1(mcr1)	5	CSB-CF745804ENL
Mcu	Recombinant Mouse Calcium uniporter protein, mitochondrial(Mcu)	2	CSB-CF668882MO
MGST2	Recombinant Human Microsomal glutathione S-transferase 2(MGST2)	3	CSB-CF013792HU
MT-ND1	Recombinant Lampetra fluviatilis NADH-ubiquinone oxidoreductase chain 1(MT-ND1)	8	CSB-CF015076LNM(A4)
NOX1	Recombinant Human NADPH oxidase 1(NOX1)	6	CSB-CF015959HU
NOX4	Recombinant Human NADPH oxidase 4(NOX4)	6	CSB-CF015961HU
NOX4	Recombinant Pongo abelii NADPH oxidase 4(NOX4)	6	CSB-CF015961PYX
ompA	Recombinant Escherichia coli Outer membrane protein A(ompA)	8	CSB-CF364256ENV(A4)
OPN3	Recombinant Human Opsin-3(OPN3)	7	CSB-CF872435HU
OR13A1	Recombinant Human Olfactory receptor 13A1(OR13A1)	7	CSB-CF854141HU
OR1A1	Recombinant Human Olfactory receptor 1A1(OR1A1)	7	CSB-CF865201HU
OR5AL1	Recombinant Human Olfactory receptor 5AL1(OR5AL1)	7	CSB-CF315097HU
OR5V1	Recombinant Human Olfactory receptor 5V1(OR5V1)	7	CSB-CF871401HU
Otop1	Recombinant Mouse Otopetrin-1(Otop1)	12	CSB-CF772148MO(A4)
P2RY12	Recombinant Human P2Y purinoceptor 12(P2RY12)	7	CSB-CF861997HU
p30	Recombinant Mycoplasma pneumoniae P30 adhesin(p30)	2	CSB-CF301931MLW
PERP	Recombinant Human p53 apoptosis effector related to PMP-22(PERP)	4	CSB-CF839325HU
PLP1	Recombinant Human Myelin proteolipid protein(PLP1)	4	CSB-CF018202HU
Pmp22	Recombinant Rat Peripheral myelin protein 22(Pmp22)	4	CSB-CF018241RA
Ptges	Recombinant Mouse Prostaglandin E synthase(Ptges)	4	CSB-CF018976MO
PVRIG	Recombinant Human Transmembrane protein PVRIG(PVRIG)	3	CSB-CF721905HU
RHD	Recombinant Human Blood group Rh(D) polypeptide(RHD)	11	CSB-CF019677HU(A4)
RNASEK	Recombinant Human Ribonuclease kappa(RNASEK)	2	CSB-CF761293HU
RNF5	Recombinant Human E3 ubiquitin-protein ligase RNF5(RNF5)	2	CSB-CF857879HU
SCD	Recombinant Human Acyl-CoA desaturase(SCD)	4	CSB-CF020802HU
SGMS1	Recombinant Human Phosphatidylcholine:ceramide cholinephosphotransferase 1(SGMS1)	5	CSB-CF801243HU
SLC30A8	Recombinant Human Zinc transporter 8(SLC30A8)	6	CSB-CF818247HU
Slc30a8	Recombinant Mouse Zinc transporter 8(Slc30a8)	6	CSB-CF807333MO
SLC31A1	Recombinant Human High affinity copper uptake protein 1(SLC31A1)	3	CSB-CF021575HU
SLC7A11	Recombinant Human Cystine/glutamate transporter(SLC7A11)	12	CSB-CF892171HU(A4)
STEAP1	Recombinant Human Metalloreductase STEAP1(STEAP1)	6	CSB-CF890691HU
STEAP2	Recombinant Human Metalloreductase STEAP2(STEAP2)	6	CSB-CF854119HU
Sting1	Recombinant Mouse Stimulator of interferon genes protein(Sting1)	4	CSB-CF023754MO
Trpc1	Recombinant Mouse Short transient receptor potential channel 1(Trpc1)	6	CSB-CF720236MO
TSPO	Recombinant Pig Translocator protein(TSPO)	5	CSB-CF764926PI
Tspo	Recombinant Mouse Translocator protein(Tspo)	5	CSB-CF025168MO
TSS56	Recombinant Orientia tsutsugamushi 56 KDA type-specific antigen	2	CSB-CF327749OCJ
U22	Recombinant Human herpesvirus 6B Protein U22(U22)	2	CSB-CF889529HKA
UGCG	Recombinant Human Ceramide glucosyltransferase(UGCG)	5	CSB-CF619088HU
VACWR074	Recombinant Vaccinia virus Protein I5(VACWR074)	2	CSB-CF318298VAI
yopB	Recombinant Yersinia enterocolitica Protein YopB(yopB)	2	CSB-CF334228YAQ

In the buffer containing detergents, most membrane proteins can maintain stability and activity. However, a few membrane proteins that are sensitive to detergents are not suitable for buffering conditions containing detergents, which are specifically incapable of purification and poor stability (easy to be degraded or obvious precipitation) and no activity. For this type of membrane protein, we suggest to try the nanodiscs technology.

In this platform, **CUSABIO uses in vitro E.coli expression system for the target protein expression**, the other expression systems such as mammalian cell system is also suitable, but we haven't developed yet. In the process of protein expression in the in vitro E.coli system, membrane skeleton protein (MSP) and phospholipid molecule (DMPC) were added to assemble nanodiscs during expression. Components: target protein (transmembrane protein), membrane skeleton protein (MSP), phospholipid molecule (DMPC).

## Advantages

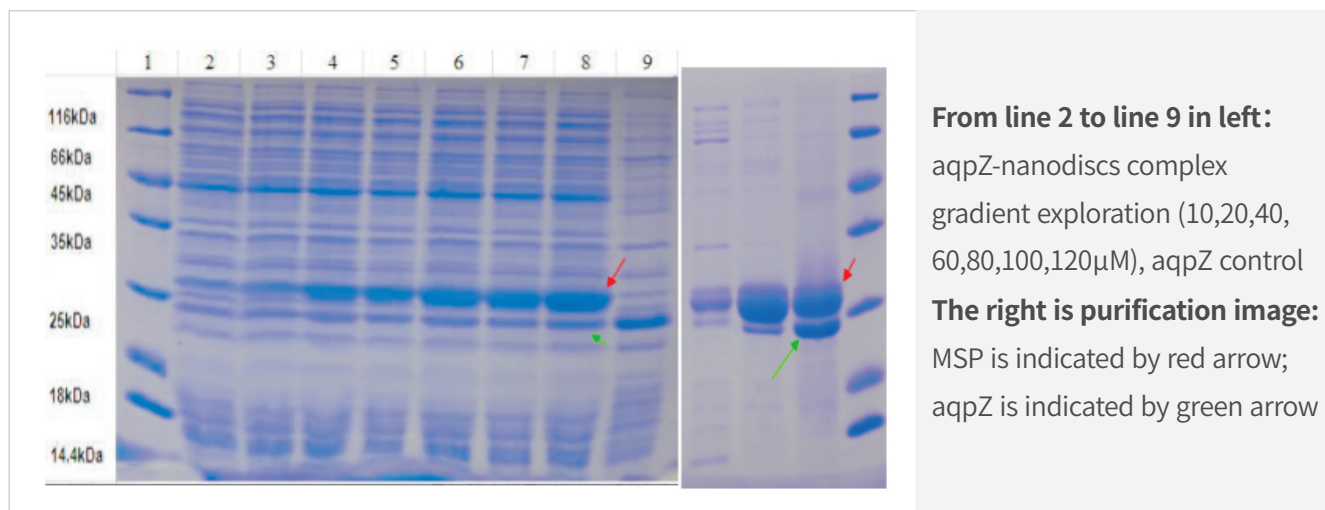
- Display natural conformation with complete biological activity.
- Detergent-free, suitable for experiments that interfere with detergents.
- Can be used directly for phage screening (provide empty nanodisc for reverse screening).
- Can be used for immunization/ELISA/SPR/BLI/cell assays.



## Case Display

### aqpZ-Nanodisc (6 times transmembrane) (CSB-CF352724ENV-N)

Validated Data : Molecular Weight and Purity Determined by SDS-PAGE



## Other Proteins Developed by Nanodisc Platform

Target	Product Name	Transmembrane Times	Code
ADTRP	Recombinant Human Androgen-dependent TFPI-regulating protein(ADTRP), Nanodisc	6	CSB-CF846640HUb1-N
Igt	Recombinant Lactococcus lactis subsp. lactis Prolipoprotein diacylglycerol transferase(Igt),Nanodisc	5	CSB-CF875009LNG-N
IspA	Recombinant Lactococcus lactis Lipoprotein signal peptidase(IspA), Nanodisc	3	CSB-CF2619Ba-N

Contact us:



Tel.: 915 515 403

Fax: 914 334 545

e-mail: [info@bionova.es](mailto:info@bionova.es)

[www.bionova.es](http://www.bionova.es)