

PD-1 mRNA

Cat. No.: LMP018

01/ Description

Programmed cell death protein 1 (PD-1), also known as CD279, is a monomeric glycoprotein composed of 288 amino acids with an approximate molecular weight of 50 kDa. It is a surface receptor expressed on activated T cells. PD-1 is primarily expressed on activated T cells and B cells and functions as a critical immune inhibitory molecule. It regulates the immune system by downregulating immune responses against host cells and suppressing T-cell inflammatory activity, thereby promoting self-tolerance and helping to prevent autoimmune diseases. Blocking the interaction between PD-1 and its ligands can expose tumor cells to immune-mediated killing. Therefore, PD-1 is an important therapeutic target in cancer treatment.

Traditional antibodies targeting multi-pass transmembrane proteins are challenging to develop due to the structural complexity of the antigen and difficulties in protein expression.

This product is a mature PD-1 mRNA containing a 5' Cap 1 structure and a 3' poly(A) tail, and is encapsulated in LNPs for efficient delivery. It enables expression of the full-length PD-1 protein, effectively overcoming the challenges associated with expressing complex transmembrane target proteins. The product can be directly used for animal immunization.

mRNA-based immunization strategies offer several advantages, including improved safety, protein expression that more closely resembles the native structure, rapid sequence optimization, and shorter production timelines.

02/ Features

1. The Cap 1 structure is more suitable for mammalian systems and provides higher translation efficiency. The addition of a poly(A) tail suppresses RNA-mediated innate immune activation and enhances mRNA stability and longevity both in vitro and in vivo. The poly(A) tail also plays a critical role in improving translation initiation efficiency.
2. The experimental procedure is simple and rapid, delivering stable and highly reproducible results.
3. The mRNA transfection efficiency is consistent and reliable.

03/ Basic Information

Gene Information	Uniprot: Q15116
Species	Homo sapiens
Amino Acid Sequence	See 08/Amino Acid Sequence
mRNA Sequence Length	1408nt
Theoretical mRNA Molecular Weight	435,363.6g/mol
mRNA Modification Type	Unmodified
LNP-mRNA Concentration	0.2-0.5mg/mL

04/ Applications

PD-1 mRNA is commonly used as an immunogen for antibody development and production. It can be introduced into animals through various delivery methods, where it is expressed in host cells and translated into a protein with a complete native conformation following proper post-translational modification. This process induces a series of humoral and cellular immune responses in the animal, leading to the generation of specific antibodies against PD-1.

05/ Storage Temperature

Storage at 2-8°C. Shelf life: 6 months under recommended storage conditions.

06/ Packaging

Product Component	LMP018-U500
	Size: 500µg
PD-1 mRNA	500µg

07/ Expression Validation

PD-1 is a multi-pass transmembrane protein, and its expression is typically detected using flow cytometry. The following method is for reference only (using adherent CHO-K1 cells as an example):

1. Solution preparation:

The basal medium is F12 medium supplemented with 10% FBS. Mix 5 mL of fetal bovine serum (FBS) with 45 mL of DMEM medium, filter sterilize through a 0.22 µm membrane, and store at 4°C (use within 2 weeks).

2. Cell culture:

- Preparation: Place trypsin at room temperature for 1–2 hours. Preheat basal medium and PBS in a 37°C water bath for 1–2 hours.
- Observation: Examine cells in the culture flask under a microscope to ensure they are in normal condition and have reached 70–90% confluence.
- Detachment: Discard the culture medium, rinse once with PBS (3 mL for a T75 flask, 1 mL for a T25 flask). Remove PBS, add trypsin (3 mL for T75, 1 mL for T25), and incubate in a 37°C constant temperature incubator for 1 minute (digestion time is typically 1–10 minutes; actual time varies depending on cell line).
- Stop digestion: Add basal medium (6 mL for T75, 2 mL for T25). Gently pipette to break up cells into a single-cell suspension. Transfer approximately 100–300 µL of the cell suspension to an EP tube.
- Cell counting: Transfer 20 µL of cell suspension to an EP tube, add 20 µL of 0.2% trypan blue, mix well, then immediately load 20 µL into the counting chamber. Place into the cell counter and take readings from three fields of view.
- Plating: Dilute cells to an appropriate density using basal medium (recommended density for CHO-K1: 2.5×10^5 cells/mL; actual density may vary by cell line). Add cell suspension to a 24-well plate, 500 µL per well. Gently shake the plate in all directions to distribute cells evenly, then culture overnight.

3. LNP-mRNA cell transfection (using one well of a 24-well plate as an example):

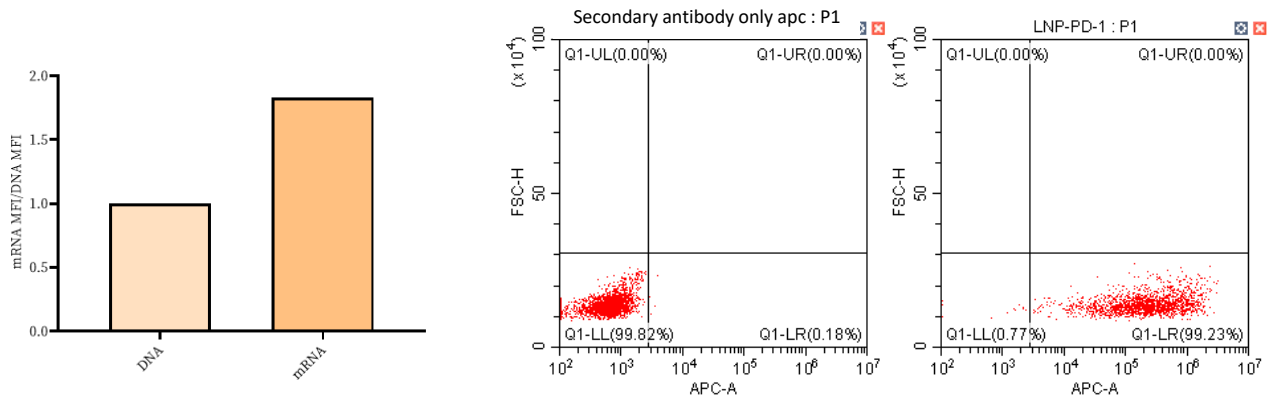
- Observation: Check cells under a microscope to ensure they are healthy and have reached ~70% confluence.
- Medium change: Replace with fresh basal medium before transfection.
- Transfection: Dilute 1 µg LNP-mRNA in 50 µL Opti-MEM reduced-serum medium, mix by pipetting. Slowly add the 50 µL diluted encapsulated sample dropwise into the cell suspension, mix gently, and incubate at 37°C for 24 hours.

4. Expression verification:

- Detach cells with trypsin 24 hours post-transfection. Add complete medium to neutralize trypsin, centrifuge at 400×g for 5 minutes, discard supernatant. Wash once with 1× PBS, vortex for 8 seconds, centrifuge at 400×g for 5 minutes, discard supernatant.
- Resuspend the cell pellet in 150 µL medium and split equally into three EP tubes. Add 100 µL of diluted positive control antibody (2 µg/mL) to one tube, and 100 µL of 1× PBS to each of the other two tubes. Vortex to resuspend cells and incubate at 4°C for 1 hour.

- c) Wash cells: Add 400 μ L 1 $\times$ PBS, centrifuge at 400 $\times$ g for 5 minutes, discard supernatant.
- d) Add flow cytometry antibody: Add 100 μ L of diluted flow antibody per well (dilution ratio according to the manufacturer's instructions). Vortex to resuspend cells and incubate at 4°C for 1 hour.
- e) Wash cells: Repeat step c.
- f) Flow cytometry detection: Add 300 μ L 1 $\times$ PBS, vortex to resuspend cells, then analyze by flow cytometry.

5. Detection Result:



Twenty-four hours after transfection with PD-1 mRNA, the percentage of PD-1-positive cells detected by a validated positive antibody exceeds 90%. The expression efficiency is higher than that achieved with DNA-based transfection.

08/ Amino Acid Sequence

MQIPQAPWPVWVAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPFE
DRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRAGQFQTL
VVGVGGLLSLVLLVWVLAIVCSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPS
GMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

09/ Related Product

Cat. No.	Product Name	Cat. No.	Product Name
GMP-M062	Vaccinia Capping System, GMP Grade	GMP-E121	T7 RNA Polymerase, GMP Grade
GMP-M072	mRNA Cap 2'-O-Methyltransferase, GMP Grade	GMP-E125	RNase Inhibitor, GMP Grade
MR016	hEPO mRNA (N1-Me-Pseudo UTP)	GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade
MR015	OVA mRNA (N1-Me-Pseudo UTP)	GMP-E131	T7 RNA Transcription Enzyme Mix, GMP Grade
LMP001	GPRC5D mRNA	LMP002	CCR7 mRNA
LMP004	CCR4 mRNA	LMP003	GPR20 mRNA
LMP005	CCR8 mRNA	LMP006	CXCR4 mRNA
LMP008	C5AR1 mRNA	LMP007	CXCR5 mRNA
LMP009	ADCYAP1R1 mRNA	LMP010	CB1 mRNA
LMP011	ETRA mRNA	LMP012	Claudin 18.2 mRNA
LMP014	CLDN9 mRNA	LMP015	SLC1A5 mRNA
LMP016	FPN1 mRNA	LMP017	CD20 mRNA
LMP013	CLDN6 mRNA	LMP038	STEAP1 mRNA
LMP039	STEAP2 mRNA		