

Version: 01

DATASHEET

Update: 06/06/2023

GMP Anti-Human CD28 Antibody (F105), mAb, Mouse

Cat. No.: GMPA02213

GMP Anti-Human CD28 Antibody (F105), mAb, Mouse can be utilized as ancillary materials for Cell, Gene, and Tissue-Based Products and are manufactured under the standards listed below:

- *USP <1043>. Ancillary materials for Cell, Gene and Tissue-engineered products*
- *ICH Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients*
- *NMPA: Technical guidelines for pharmaceutical research and evaluation of immune cell therapy products*
- *Guideline and procedure specified in Chinese Pharmacopeia*

Product Overview

Specificity	This product is specific for Human CD28
Host Species	Mouse
Immunogen	Recombinant Human CD28
Conjugation	Unconjugated
Recommended Applications	<i>ex vivo</i> cell culture process; Flow cytometry

Product Properties

Form	Liquid
Storage Buffer	Supplied in 10 mM Sodium Citrate, 150 mM NaCl, pH 6.5.
Concentration	5 mg/ml
Storage Instructions	The product remains stable at -70 ± 10 °C. Avoid repeated freeze-thaw cycles. Please refer to the COA for specific expiry date.
Isotype	Mouse IgG1 κ
Clonality	Monoclonal
Clone ID	F105

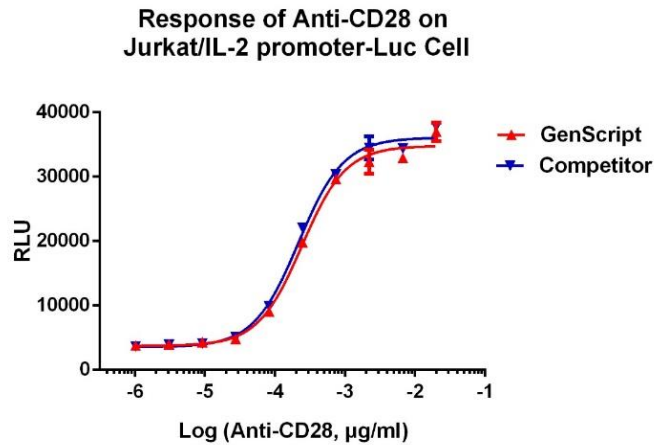
Protocol of T cell Activation using Anti-CD3 and Anti-CD28 Antibodies

The following protocol can be a starting point for T cell activation; the actual result can be influenced by many factors, such as cell sample condition, personnel operation, experimental reagents, etc. Further optimization might be needed for optimal performance.

Operations	Time	Description	Key Point
Step 1: Anti-CD3 coating	Day 1	a) Prepare the appropriate amount of anti-CD3 (Cat. No. GMPA02199) working solution with sterile DPBS as needed. b) Take out a 96-well culture plate and aspirate 100 μ l of anti-CD3 working solution into the wells for the experiment. Place the plate at 4 °C for overnight. Note: The wells in the edge of the plate are not suggested for the experiment to avoid edge effects.	The recommended concentration of anti-CD3 working solution is 5-20 μ g/ml
Step 2: T cell preparation	Day 2	a) Pan T cells are isolated from prepared PBMCs and resuspended in culture media. Adjust the concentration of cell suspension to 1.0×10^6 cells/ml. b) Aspirate an appropriate amount of anti-CD28 antibody (Cat. No. GMPA02213) into the cell suspension to make it at an optimal concentration.	<ul style="list-style-type: none"> • Cell viability (%): $\geq 90\%$ • The recommended concentration of anti-CD28 antibody in cell suspension is 5-50 μg/ml
Step 3: Wash		a) Take out the pre-coated 96-well plate from Step 1. b) Aspirate and remove the liquid in the wells, then add 200 μ l of sterile DPBS to each well slowly. c) Repeat the wash step described in "b)" for three times.	/
Step 4: T cell activation		a) After finishing the wash, add 200 μ l of cell suspension (contains 2.0×10^5 cells) from Step 2 into each well. b) And add 200 μ l of sterile DPBS to the wells on the edge of the plate to avoid the edge effects of the assay caused by the heavy evaporation of the experimental wells. c) Put the plate to a cell culture incubator and incubate for 72 h.	<ul style="list-style-type: none"> • Cell number: 2.0×10^5 cells/well • Activation time: 72 h

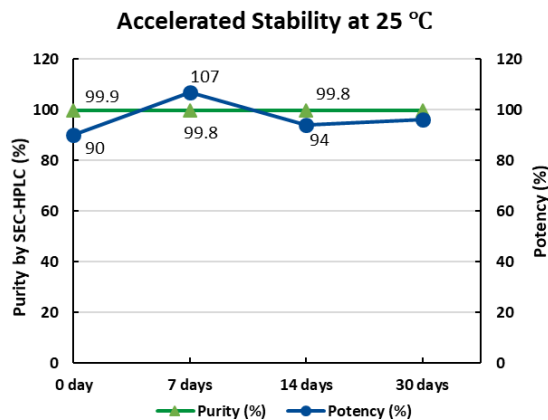
Data Images

1. Signal Resonse in Reporter Cell Line

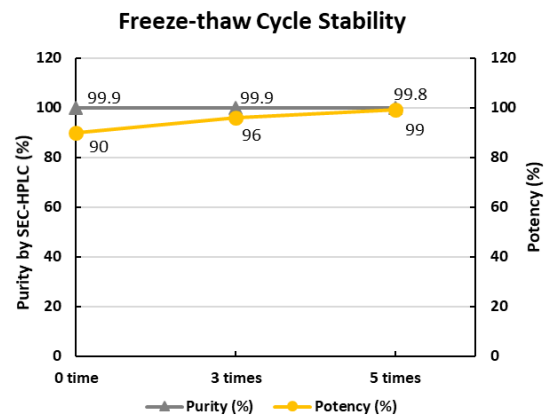


Jurkat/IL-2-Luc Promoter cells were co-stimulated with a serial of concentrations of GMP Anti-Human CD28 Antibody (F105), mAb, Mouse (Cat. No. [GMPA02213](#)) and GMP Anti-Human CD3 Antibody (OKT3), mAb, Mouse (Cat. No. [GMPA02199](#)) for 6 h. Receptor-mediated signaling induced luminescence via activation of the IL-2 pathway.

2. High Stability



Accelerated stability test of GMP Anti-Human CD28 Antibody (F105), mAb, Mouse (Cat. No. [GMPA02213](#)) at 25 °C showed that this product is stable at 25 °C for 30 days and maintained high purity and potency.



Freeze-thaw cycle stability test of GMP Anti-Human CD28 Antibody (F105), mAb, Mouse (Cat. No. [GMPA02213](#)) showed that this product is stable for 5 times of freeze-thaw cycles and maintained high purity and potency.

Quality Control Specifications

GMP Anti-Human CD28 Antibody (F105), mAb, Mouse (Cat. No. **GMPA02213**) is manufactured in compliance with GMP quality management system standards and with stringent process controls and complete documentation records. It meets the following quality control specifications.

Test Items	Specifications
Appearance	Clear, colorless liquid
pH	6.5 ± 0.5
Concentration	5.0 ± 0.5 mg/ml tested by A280 nm
Purity	≥ 95% as analyzed by SEC-HPLC
Sequence	The coverage of the light chain and heavy chain sequence was 100% consistent with the theoretical amino acid sequence by mass spectrometry (MS)
Residual HCP	≤ 100.0 ng/mg by ELISA method
Residual HCD	≤ 10.0 pg/mg by quantitative PCR method
Residual Protein A	≤ 10.0 ng/mg by ELISA method
Endotoxin Level	< 0.1 EU/mg by gel clotting method
Sterility	Sterile
Reactivity	Qualified

Target Background

CD28 (Cluster of Differentiation 28) is one of the proteins expressed on T cells that provide co-stimulatory signals required for T cell activation and survival. It plays an important role in cell proliferation and cytokine production and promotion of T-cell survival. It also enhances the production of IL-4 and IL-10 in T cells in conjunction with TCR/CD3 ligation and CD40L co-stimulation.

For laboratory research and *ex vivo* cell isolation and culture applications. Direct human use, including taking orally and injection and clinical use are forbidden.

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