Add&Read Mouse Fc Kit

DD2105

Vazyme

Instruction for Use

Version 24.1

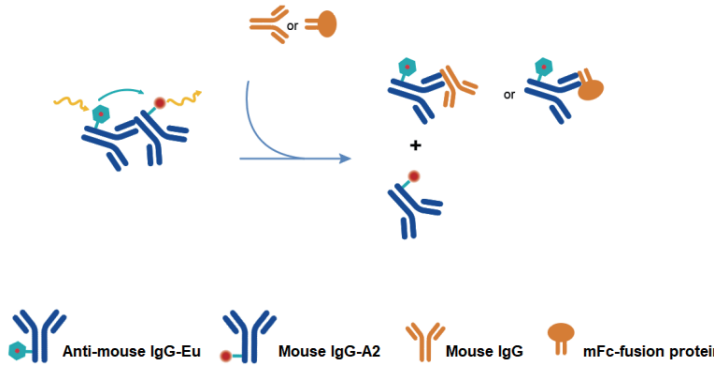
Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

The Mouse Fc Kit can be used for high-throughput detection of the concentration for Mouse IgG or mFc fusion protein in cell supernatant or after purification. There is an antibody strain in the reagent kit that recognizes the Fc region of Mouse IgG and labels the fluorescent donor Eu (Anti-Mouse IgG-Eu); There is also a Mouse IgG labeled with fluorescent receptor A2 (Mouse IgG-A2).

In the solution, Anti-Mouse IgG-Eu binds to Mouse IgG-A2, and 320 nm light excites the fluorescent donor Eu, which emits 620 nm light, which excites the fluorescent receptor A2, which emits 665 nm light, which is a phenomenon of Fluorescence Resonance Energy Transfer (FRET) . When adding Mouse IgG or mFc-fusion protein to the solution, it will compete to bind Anti-Mouse IgG-Eu and destroy FRET phenomenon. The concentration of Mouse IgG or mFc-fusion protein to be measured is inversely proportional to the signal value of FRET (light intensity ratio of 665 nm/620 nm) .



02/Product Components

Components	DD2105-00(100 tests)	DD2105-01(500 tests)	DD2105-02(10,000 tests)
IgG Standard	1 vial	1 vial	2 vial
Anti-mouse IgG-Eu	10 μl	50 μl	1 ml
Mouse IgG-A2	10 μl	50 μl	1 ml
Diluent	4 ml	20 ml	20 ml
Detection Buffer	4 ml	7 ml	105 ml

03/Storage Conditions

Mouse Fc Kit is stored at -30 ~ -15 ℃ and transported at ≤ 0 ℃;

The freeze-dried powder of IgG standard is stored at 2 ~ 8 ℃, dissolved by adding ddH_2O, and then stored separately at -30 ~ -15 ℃ to avoid repeated freezing and thawing.

Anti-Mouse IgG-Eu and Mouse IgG-A2 are stored at -30 ~ -15 ℃ to avoid repeated freezing and thawing.

Diluent and Detection Buffer are stored at -30~-15 ℃.

04/Scope of Application

Cell Supernatant, Purified Protein

05/Self-provided Materials

96/384 Shallow Well Plate
Microplate Reader (with HTRF/TR-FRET module)

06/Precautions

1. Anti-Mouse IgG-Eu and Mouse IgG-A2 are suggested to be stored at -30 ~ -15 ℃ under the condition of storage solution (50×) to avoid repeated freezing and thawing.
2. The IgG Standard is dissolved in ddH_2O and then stored at -30 ~ -15 ℃ separately to avoid repeated freezing and thawing.

07/Experiment Process

07-1/Reagent Preparation

1. Preparation of Anti-Mouse IgG-Eu and Mouse IgG-A2 working solutions (which is 50× storage solution)
   The reaction volume of 96/384 shallow well plate is 20 μl, and it is suggested that 5 μl of Anti-Mouse IgG-Eu and 5 μl Mouse IgG-A2 working solution should be added to each 20 μl system. Before preparation, calculate the volume of Anti-Mouse IgG-Eu and Mouse IgG-A2 needed in this experiment: V= (number of wells × 5/50) μl.

   a. Preparation of Anti-Mouse IgG-Eu working solution;
      ● Take the Anti-Mouse IgG-Eu out of the refrigerator, and leave it at room temperature to dissolve it into 50× storage solution.
      ● Add 49 volumes of Detection Buffer (49V μl) to 1 volume of Anti-Mouse IgG-Eu (V μl) and mix evenly.

   b. Preparation of Mouse IgG-A2 working solution:
      ● Take the Mouse IgG-A2 out of the refrigerator and let it dissolve at room temperature, which is 50× storage solution.
      ● Add 49 volumes of Detection Buffer (49V μl) to 1 volume of Mouse IgG-A2 (V μl) and mix evenly.

      ▲ Anti-Mouse IgG-Eu and Mouse IgG-A2 are suggested to be stored at -30 ~ -15 ℃ under the condition of storage solution (50×) to avoid repeated freezing and thawing.

1. Preparation of IgG Standard

The reaction volume of 96/384 shallow well plate is 20 μl, and each well needs 10 μl of IgG Standard. Calculate the required IgG standard volume before preparation. By following the steps below to prepare, 100 μl IgG Standard can be obtained.

a. Add 2.5 ml ddH_2O to the schering bottle of IgG Standard, and after it is fully dissolved, it is Std 11.

b. Take 50 μl of Std 11, add 100 μl Diluent, and mix evenly to obtain Std 10.

c. In the same way, the gradient dilution of 3 times was carried out to obtain Std 9-Std 1.

Standard	Dilution Method	mIgG Concentration (μg/ml)
Std 11	-	324
Std 10	50 μl Std 11+ 100 μl Diluent	108
Std 9	50 μl Std 10+ 100 μl Diluent	36
Std 8	50 μl Std 9 + 100 μl Diluent	12
Std 7	50 μl Std 8 + 100 μl Diluent	4
Std 6	50 μl Std 7 + 100 μl Diluent	1.3333
Std 5	50 μl Std 6 + 100 μl Diluent	0.4444
Std 4	50 μl Std 5 + 100 μl Diluent	0.1481
Std 3	50 μl Std 4 + 100 μl Diluent	0.0494
Std 2	50 μl Std 3 + 100 μl Diluent	0.0165
Std 1	50 μl Std 2 + 100 μl Diluent	0.0055
Std 0	100 μl Diluent	0

▲ The IgG Standard is dissolved in ddH_2O and then stored at -30 ~ -15 ℃ separately to avoid repeated freezing and thawing.

07-2/Sample Preparation

Samples were diluted with Diluent or freshly prepared buffer with 0.5% BSA and pH 7.0.

07-3/Reaction System

1. Sample Adding

The reaction volume of 96/384 shallow well plate is 20 μl, and samples are added according to the experimental grouping and reaction system in the table below.

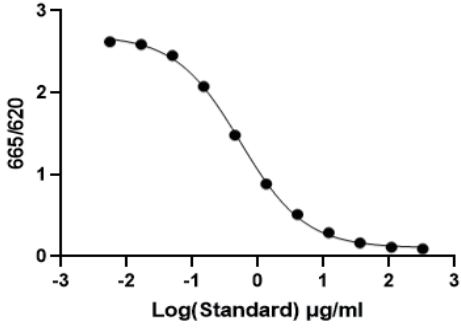
	Standard/Sample	Positive Control	Negative Control	Buffer Control
IgG Standard/Sample	10 μl	-	-	-
Mouse IgG-A2	5 μl	5 μl	-	-
Anti-Mouse IgG-Eu	5 μl	5 μl	5 μl	-
Diluent	-	10 μl	10 μl	10 μl
Detection Buffer	-	-	5 μl	10 μl

2. The order of reagent addition is:
   a. Add 10 μl IgG Standard/ sample to 96/384 shallow well plate.
   b. Add 5 μl Mouse IgG-A2, and gently mix twice in the sample adding well with a pipette.
   c. Add 5 μl of anti-mouse IgG-Eu, and gently mix twice in the sample adding well with a pipette. (This reagent is added last) . Incubate at room temperature or 25 ℃ for 2 hours, and detect with microplate reader (equipped with HTRF module) . The excitation light is 320 nm, and the emitted light with two wavelengths (665 nm and 620 nm) is detected.

08/Data Processing

The fluorescence value of 665 nm is divided by the fluorescence value of 620 nm to obtain a value of 665/620 . With $\log_{10}$ [sample concentration] as the abscissa and the value of 665/620 as the ordinate, the curve was made by curve fitting.

Std No.	[Standard], μg/ml	665/620	CV
Std 11	324	0.0939	0.8%
Std 10	108	0.1133	2.3%
Std 9	36	0.1653	0.9%
Std 8	12	0.2912	1.8%
Std 7	4	0.5131	0.9%
Std 6	1.3333	0.8872	1.2%
Std 5	0.4444	1.4813	2.3%
Std 4	0.1481	2.0763	2.0%
Std 3	0.0494	2.4537	1.8%
Std 2	0.0165	2.5885	0.9%
Std 1	0.0055	2.6207	0.6%
Positive control	0	2.6781	1.2%
Negative control	-	0.0840	0.0%



Vazyme Biotech Co.,Ltd.
www.vazyme.com
400-007-8058 (China) +86 400-168-5000 (Global)
support@vazyme.com