Add&Read
Human Fc Kit (broad range)

DD2103

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Instruction for Use

Version 24.1

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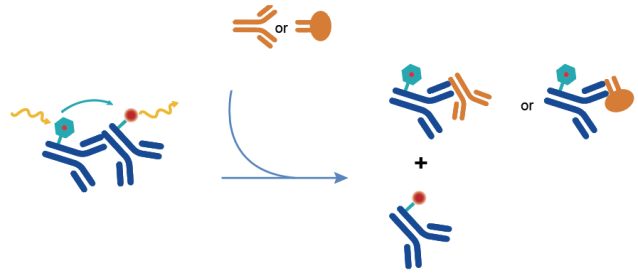
Contents

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

01/Product Description

Human Fc Kit (broad range) can be used to detect the concentration of Human IgG or hFc-fusion protein in cell supernatant or after purification by high throughput. There is an antibody strain in the reagent kit that recognizes the Fc region of Human IgG and labeled with the fluorescent donor Eu (Anti-human IgG-Eu); There is also Human IgG, labeled with fluorescent receptor A2 (Human IgG-A2).

In the solution, Anti-human IgG-Eu binds to Human IgG-A2, and 320 nm light excites the fluorescent donor Eu,, and the Eu donor emits 620 nm light, 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 Human IgG or hFc-fusion protein to the solution, it will compete to bind Anti-human IgG-Eu and destroy FRET phenomenon. The concentration of Human IgG or hFc-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	DD2103-00(100tests)	DD2103-01(500 tests)	DD2103-02(10,000 tests)
IgG Standard	1 vial	1 vial	2 vial
Anti-human IgG-Eu	10 μl	50 μl	1 ml
Human 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

The Human Fc Kit (broad range) 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-human IgG-Eu and Human IgG-A2 are stored at -30~-15℃ to avoid repeated freezing and thawing.
Diluent and Detection Buffer are stored at -20~4℃.

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-human IgG-Eu and Human 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-human IgG-Eu and Human 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 to add 5 μl of Anti-Human IgG-Eu and 5 μl Human IgG-A2 working solution into every 20 μl system. Calculate the volumes of Anti-human IgG-Eu and Human IgG-A2 required for this experiment before preparation: V= (Number of wells ×5/50) μl

   a. Preparation of Anti-human IgG-Eu working solution;
      - Take the Anti-human IgG-Eu 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 Anti-human IgG-Eu (V μl) and mix evenly.

   b. Preparation of Human IgG-A2 working solution:
      - Take Human 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 Human IgG-A2(V μl) and mix evenly.

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

2. 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. According to the following steps, 100 μl IgG Standard can be obtained.

   - Add 1.1 ml ddH_2O to the schering bottle of IgG Standard, and after it is fully dissolved, it is Std 11.
   - Take 50 μl of Std 11, add 100 μl Diluent, and mix evenly to obtain Std 10.
   - In the same way, the gradient dilution of 3 times was carried out to obtain Std 9-Std 1.

Standard	Dilution Method	hIgG concentration, μg/ml
Std 11	-	2916
Std 10	50 μl Std 11 + 100 μl Diluent	972
Std 9	50 μl Std 10 + 100 μl Diluent	324
Std 8	50 μl Std 9 + 100 μl Diluent	108
Std 7	50 μl Std 8 + 100 μl Diluent	36
Std 6	50 μl Std 7 + 100 μl Diluent	12
Std 5	50 μl Std 6 + 100 μl Diluent	4
Std 4	50 μl Std 5 + 100 μl Diluent	1.333
Std 3	50 μl Std 4 + 100 μl Diluent	0.444
Std 2	50 μl Std 3 + 100 μl Diluent	0.148
Std 1	50 μl Std 2 + 100 μl Diluent	0.049
Std 0	100 μl Diluent	0

▲ The IgG Standard is dissolved in ddH2O 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.If the concentration of the sample to be tested is between 0.049-972 μg/ml, 10 μl of supernatant can be directly taken for detection.

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	-	-	-
Human IgG-A2	5 μl	5 μl	-	-
Anti-human 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:

- Add 10 μl IgG Standard/sample to 96/384 shallow well plate.
- Add 5 μl of human IgG-A2, and gently mix twice in the sample adding well with a pipette.
- Add 5 μl of Anti-Human IgG-Eu, and gently mix it 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/TR-FRET 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 log10 [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
Std 11	2916	0.1077
Std 10	972	0.1308
Std 9	324	0.1890
Std 8	108	0.3861
Std 7	36	0.7757
Std 6	12	1.5466
Std 5	4	2.3356
Std 4	1.333	2.9276
Std 3	0.444	3.3311
Std 2	0.148	3.4611
Std 1	0.049	3.5574
Positive control	0	3.5354
Negative control	-	0.0706