Add&Read
Human Fc Kit (broad range)

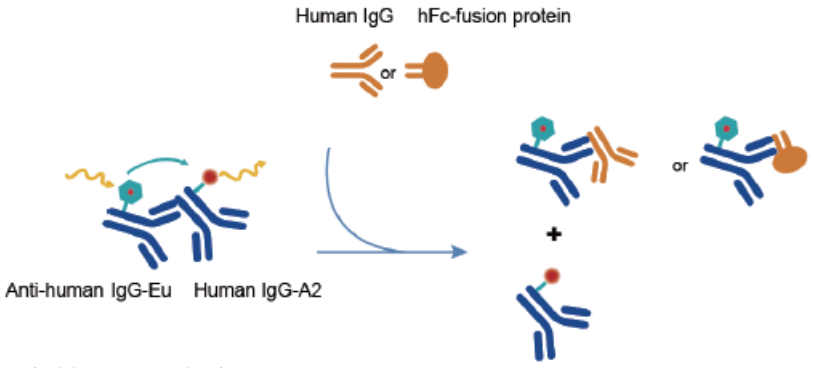
DD2103

Version 20.1

Product Description

This product can be used to measure the concentration of human IgG in cell culture supernatant, purified human IgG or hFc fusion protein. The kit contains anti-human IgG-Eu that is labeled with a fluorescent donor Eu and recognizes the Fc region of human IgG. It also contains human IgG labeled with a fluorescent acceptor A2 (human IgG-A2).

The anti-human IgG-Eu and human IgG-A2 bind to each other in solution, and when excited at 320 nm, the fluorescent donor Eu emits 620 nm of fluorescence that in turn excites the fluorescent acceptor A2. This acceptor A2 then emits at 665 nm, and this phenomenon is called fluorescence resonance energy transfer (FRET). When a sample of a human IgG or hFc-fusion protein is added to the solution, it competes to bind with the anti-human IgG-Eu, disrupting the FRET phenomenon. The concentration of the human IgG or hFc-fusion protein in the sample is inversely proportional to the FRET signal (665 nm/620 nm fluorescence intensity ratio).



Product Component (500 tests, 50 ×)

Name	IgG Standard	Anti-human IgG-Eu	Human IgG-A2	Diluent	Detection buffer
Specification	1 vial	1 ml / vial	1 ml / vial	20 ml/vial	105 ml/vial
Storage	2 ~ 8℃	≤ -20℃	≤ -20℃	-20 ~ 4℃	-20 ~ 4℃

* The diluent and detection buffer are shipped frozen and can be stored at 2 ~ 8°C after use.

Reagent Preparation

1. Preparing the Antibody Working Solutions

The reaction volume of a 384-shallow well plate is 20 µl/well, and each well requires 5 µl of antibody. Before preparing the solutions, calculate the volume of reagents needed in the test while considering the standard curve and the number of test samples. For other plates, calculate based on the required volume.

V (volume of antibody to be diluted) = (number of wells × 5/50) µl

Preparing the anti-human IgG-Eu working solution:

- Thaw the anti-human IgG-Eu at room temperature.
- To every 1 part of anti-human IgG-Eu (V µl), add 49 parts of detection buffer (49 × V µl) and mix thoroughly.

Preparing the human IgG-A2 working solution:

- Thaw the human IgG-A2 at room temperature.
- To every 1 part of human IgG-A2 (V µl), add 49 parts of detection buffer (49 × V µl) and mix thoroughly.

▲ Anti-human IgG-Eu and human IgG-A2: It is recommended to aliquot (50 ×) and store at -20°C or -70°C. Avoid repeated freezing and thawing.

2. Preparing the IgG Standard

The reaction volume of a 384-shallow plate is 20 µl/well, and each well requires 10 µl of IgG Standard. Calculate the volume of IgG Standard needed before preparation. For other plates, calculate based on the required volume.

Follow the steps below to prepare 100 µl of IgG Standard.

- Add 1.1 ml of ddH2O to a vial of IgG Standard. After it is well dissolved, use this solution as Std 11.
- Add 100 µl of diluent to 50 µl of Std 11. Mix thoroughly to obtain Std 10.
- Perform three-fold dilution using the same method to obtain Std 9 to 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

▲ IgG standard: It is recommended to aliquot the dissolved standard into 1.5 ml EP tubes and store at -20°C or -70°C. Avoid repeated freezing and thawing.

3. Sample Preparation

If the test sample concentration is 0.049 – 972 μg/ml, use 10 μl of the supernatant directly for the test.

Experimental protocol (384-shallow well plate)

The reaction volume of a 384-shallow well plate is 20 μl/well. Follow the steps below to add the sample. Negative and positive control are required.

	Negative Control	Positive control	Buffer Control	Sample/Standard
Sample/IgG Standard	-	-	-	10 μl
Diluent	10 μl	10 μl	10 μl	-
Anti-human IgG-Eu	5 μl	5 μl	-	5 μl
Human IgG-A2	-	5 μl	-	5 μl
Detection buffer	5 μl	-	10 μl	-

Add the reagents in the following order:

- Add 10 μl of standard or sample to the 384-shallow well plate.
- Add 5 μl of human IgG-A2 and use the pipette to gently mix in the well twice.
- Add 5 μl of anti-human IgG-Eu and use the pipette to gently mix in the well twice. (Add this reagent at last)

Incubate for 2 hours at room temperature or 25°C. A microplate reader equipped with a HTRF/TR-FRET module is used to detect. Excite at 320 nm and measure at 665 nm and 620 nm.

Data Consolidation

The fluorescence at 665 nm divided by the fluorescence at 620 nm to obtain the value of 665/620. Using log10 (concentration of standard) as the x-axis and the 665/620 value as the y-axis, create the standard curve by curve fitting.

Std No.	Standard ng/ml	665/620
Negative control	Negative control	0.0706
Std 0	0	3.5354
Std 1	0.049	3.5574
Std 2	0.148	3.4611
Std 3	0.444	3.3311
Std 4	1.333	2.9276
Std 5	4	2.3356
Std 6	12	1.5466
Std 7	36	0.7757
Std 8	108	0.3861
Std 9	324	0.1890
Std 10	972	0.1308
Std 11	2916	0.1077