Add&Read Human Fc Kit

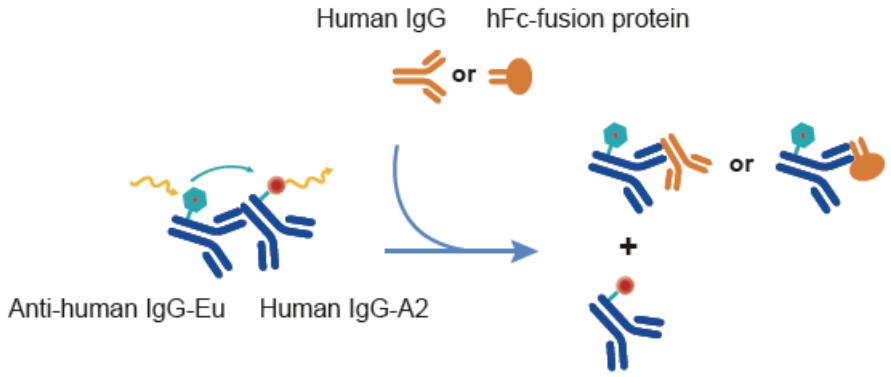
DD2102

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).



Components (500 tests, 50 ×)

Name	IgG Standard	Anti-human IgG-Eu	Human IgG-A2	Diluent	Detection buffer
Specification	lyophilized powder	50 μl/vial	50 μl/vial	20 ml/vial	7 ml/vial
Storage	2 ~ 8℃	≤ -20℃	≤ -20℃	4 ~ -20℃	4 ~ -20℃

* 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.
- Add 49 volumes of Detection buffer (49 × V μl) to 1 volume of Anti-human IgG-Eu (V μl) and mix well.

Preparing the human IgG-A2 working solution:

- Thaw the human IgG-A2 at room temperature..
- Add 49 volumes of Detection buffer (49 × V μl) to 1 volume of Anti-human IgG-A2 (V μl) and mix well.

▲ 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 system of the 384-shallow well plate is 20 μl/well and requires 10 μl of IgG Standard per well. Calculate the required volume of IgG Standard before preparation (other well plates are calculated according to the required volume). 200 μl of IgG Standard can be obtained by following the preparation steps below.

- Add 2.5 ml of ddH2O to the vial of IgG Standard and dissolve well to generate Std 9.
- Add 200 μl of Diluent to 100 μl of Std 9 and mix well to obtain Std 8.
- Perform 3-fold dilution in the same way to obtain Std 7-Std 1.

Standard	Dilution Method	Concentration of hIgG ng/ml
Std 9		36000
Std 8	100 µl Std 9 + 200 µl Diluent	12000
Std 7	100 µl Std 8 + 200 µl Diluent	4000
Std 6	100 µl Std 7 + 200 µl Diluent	1333
Std 5	100 µl Std 6 + 200 µl Diluent	444
Std 4	100 µl Std 5 + 200 µl Diluent	148
Std 3	100 µl Std 4 + 200 µl Diluent	49
Std 2	100 µl Std 3 + 200 µl Diluent	16.5
Std 1	100 µl Std 2 + 200 µl Diluent	5.5
Std 0	200 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 Dilution

Dilute samples with Diluent (DD201 or 1 × DD202) or freshly prepared buffer (pH 7.0) containing 0.5% BSA to make the concentration of the sample to be assayed within the range of 5.5-36000 ng/ml.

Experiment Process (384-shallow Well Plate)

The reaction volume of the 384-shallow well plate is 20 µl. 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 the 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 h at room temperature or 25°C and detect with a microplate reader (configured with HTRF module) with excitation light at 320 nm and emission light at two wavelengths (665 nm and 620 nm).

Data Consolidation

The fluorescence value at 665 nm divided by the fluorescence value at 620 nm to obtain the value of 665/620. Using log10 (concentration of standard) as the x-axis and the 665/620 × 10000 value as the y-axis, create the standard curve by curve fitting. (The data in the table below are obtained from the Tecan Spark microplate reader.)

Std No.	Standard ng/ml	665/620 × 10000	CV
Negative control	Negative control	664	3.2%
Std 0	0	21775	2.4%
Std 1	5.5	21732	1.2%
Std 2	16.5	20716	0.8%
Std 3	49	18964	0.5%
Std 4	148	15955	1.4%
Std 5	444	10831	2.6%
Std 6	1333	6163	1.6%
Std 7	4000	3336	1.6%
Std 8	12000	1804	1.8%
Std 9	36000	1095	1.4%