Add&Read Human IgG Kit

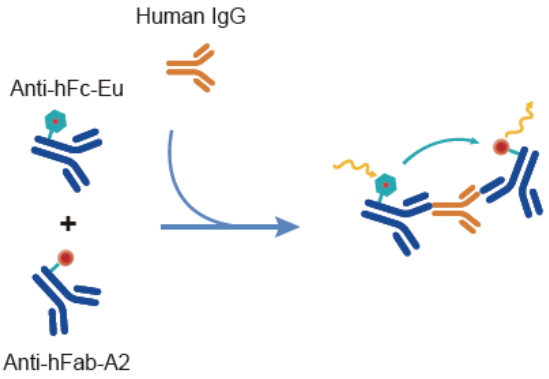
DD2101

Version 20.1

Product Overview

This product can be used to detect the concentration of human IgG in cell culture supernatant or purified IgG. There are two strains of antibodies recognizing hIgG in the kit: anti-hFc recognizing the Fc region (labeled with fluorescent donor Eu, anti-hFc-Eu); and anti-hFab recognizing the Fab region (labeled with fluorescent acceptor A2, anti-hFab-A2).

When anti-hFc-Eu and anti-hFab-A2 are in close proximity (binding interaction occurs), fluorescence resonance energy transfer (FRET) can occur. The fluorescent donor Eu is excited at 320 nm. Eu emits at 620 nm, and this 620 nm light excites the fluorescent acceptor A2, which emits at 665 nm. The concentration of the human IgG is proportional to the FRET signal value (665 nm/620 nm fluorescence intensity ratio).



Components (500 tests, 50 ×)

Name	IgG Standard	Anti-hFc-Eu	Anti-hFab-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 system for the 384-shallow well plate is 20 μl/well, and 5 μl of antibody is required for each well. 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-hFc-Eu working solution:

- Take out the anti-hFc-Eu from freezer and thaw it at room temperature.
- Add 49 volumes of Detection buffer (49 × V μl) to 1 volume of Anti-hFc-Eu (V μl) and mix well.

Preparing the anti-hFab-A2 working solution:

- Take out human anti-hFab-A2 from freezer and thaw it at room temperature.
- Add 49 volumes of Detection buffer (49 × V μl) to 1 volume of Anti-hFab-A2 (V μl) and mix well.

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

2. Preparing the IgG Standard

The reaction system for 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 ddH2O to the vial of IgG Standard and dissolve well. Add 150 μl Diluent to 150 μl of the fully dissolved IgG Standard and mix well, which is Std 8.

● Add 200 µl of Diluent to 100 µl of Std 8 and mix thoroughly to obtain Std 7.
● Perform 3-fold dilution in the same way to obtain Std 6-Std 1.

Standard	Dilution Method	Concentration of hIgG ng/ml
Std 8		2000
Std 7	100 µl Std 8 + 200 µl Diluent	666.67
Std 6	100 µl Std 7 + 200 µl Diluent	222.22
Std 5	100 µl Std 6 + 200 µl Diluent	74.07
Std 4	100 µl Std 5 + 200 µl Diluent	24.69
Std 3	100 µl Std 4 + 200 µl Diluent	8.23
Std 2	100 µl Std 3 + 200 µl Diluent	2.74
Std 1	100 µl Std 2 + 200 µl Diluent	0.91
Std 0	200 µl Diluent	0

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

3. Sample Dilution

Samples are diluted using 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 0.91-2000 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. A negative control and a fluorescent donor Eu control are required and added as shown in the table below.

	Negative Control	Eu Control	Buffer Control	Sample/Standard
Sample/IgG Standard	-	-	-	10 µl
Dilunet	10 µl	10 µl	10 µl	-
Anti-hFc-Eu	5 µl	5 µl	-	5 µl
Anti-hFab-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 anti-hFab-A2 and use the pipette to gently mix in the well twice.
● Add 5 µl of Anti-hFc-Eu and use the pipette to gently mix in the well twice.
(Same volume of Anti-hFab-A2 and Anti-hFc-Eu can be mixed and add 10 µl of the mixture to the reaction system.)
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
Std 0	0	1641	3.4%
Std 1	0.91	1999	4.9%
Std 2	2.74	2723	2.6%
Std 3	8.237	4833	0.6%
Std 4	24.697	9927	1.7%
Std 5	74.077	21370	3.3%
Std 6	222.227	33026	0.2%
Std 7	666.67	38872	0.7%
Std 8	2000	44166	0.1%