Add&Read Human IgG Kit

DD2101

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Instruction for Use

Version 24.1

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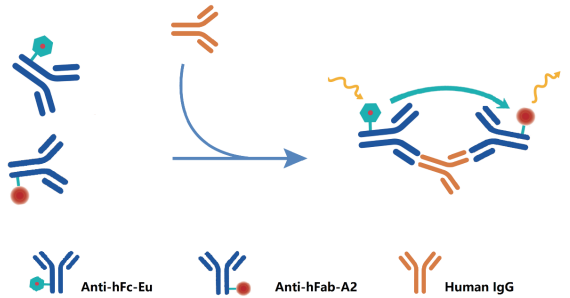
Contents

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

01/Product Overview

Human IgG Kit is designed for detecting the concentration of Human IgG in cell supernatants or after purification. The kit contains two antibodies that recognize Human IgG, which are: an antibody that recognizes the Fc region, labeled with the luminescent donor Europium (Anti-hFc-Eu); and an antibody that recognizes the Fab region, labeled with the luminescent acceptor A2 (Anti-hFab-A2).

When Anti-hFc-Eu and Anti-hFab-A2 are in close proximity (binding occurs), Fluorescence Resonance Energy Transfer (FRET) can take place. Using light at 320 nm to excite the luminescent donor Eu, Eu emits light at 620 nm, which in turn excites the luminescent acceptor A2, and A2 emits light at 665 nm. The concentration of Human IgG to be tested is directly proportional to the FRET signal value (the ratio of the light intensity at 665 nm to 620 nm).



02/Product Components

Component	DD2101-00(100 tests)	DD2101-01(500 tests)	DD2101-02(10,000 tests)
IgG Standard	1 vial	1 vial	2 vial
Anti-hFc-Eu	10 μl	50 μl	1 ml
Anti-hFab-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 IgG Kit should be stored at temperatures between -30 ~ -15℃, and transported at temperatures ≤0℃.

The IgG Standard lyophilized powder should be stored at 2 ~ 8℃. After dissolving it with ddH_2O, it should be aliquoted and stored at temperatures ranging from -30 ~ -15℃, avoiding repeated freezing and thawing. Anti-hFc-Eu and Anti-hFab-A2 should be stored at temperatures between -30 and -15℃, and should be kept away from repeated freezing and thawing.

The Diluent and Detection Buffer should be stored at temperatures between -20 ~ 4℃.

04/Applicable Scope

Cell Supernatant and Purified Protein

05/Self-provided Materials

96/384 Shallow Well Plate

The Enzyme-linked Immunosorbent Assay (ELISA) reader (equipped with HTRF/TR-FRET module).

06/Precautions

1. Anti-hFc-Eu and Anti-hFab-A2 are recommended to be aliquoted under storage conditions (50×) and stored at temperatures between -30 ~ -15℃ to avoid repeated freezing and thawing.
2. Take the IgG Standard and dissolve it in ddH_2O, then aliquot and store at temperatures between -30 to -15℃ to avoid repeated freezing and thawing.

07/Experiment Process

07-1/Reagent Preparation

1. Anti-hFc-Eu and Anti-hFab-A2 working solutions should be prepared using a storage solution at a concentration of 50×.

The reaction volume for a 96/384 shallow well plates is 20 μl, and it is recommended to add 5 μl of Anti-hFc-Eu and Anti-hFab-A2 working solutions to each 20 μl system. Before preparation, first calculate the volume of Anti-hFc-Eu and Anti-hFab-A2 required for this experiment. V= (Number of wells ×5/50) μl

◇ Anti-hFc-Eu Working Solution Preparation:

- Take the Anti-hFc-Eu out of the refrigerator and let it dissolve at room temperature, which is the 50× storage solution.
- Take 1 volume of Anti-hFc-Eu (V μl) and add 49 volumes of Detection Buffer (49V μl), then mix thoroughly.

◇ Anti-hFab-A2 Working Solution Preparation:

- Take the Anti-hFab-A2 out of the refrigerator and let it dissolve at room temperature, which is the 50× storage solution.
- Take 1 volume of Anti-hFab-A2 (V μl) and add 49 volumes of Detection Buffer (49V μl), then mix thoroughly.

▲ Anti-hFc-Eu and Anti-hFab-A2 are recommended to be aliquoted under storage conditions (50×) and stored at temperatures between -30 ~ -15℃ to avoid repeated freezing and thawing.

2. IgG Standard Configuration

The reaction volume for a 96/384 shallow well plates is 20 μl, with each well requiring 10 μl of IgG Standard. Calculate the required volume of IgG Standard before preparation. Follow these steps to prepare 200 μl of IgG Standard.

- Add 2.5 ml of ddH_2O to the vial of IgG Standard. After complete dissolution, take 150 μl of the dissolved IgG Standard and add 150 μl of Diluent, mix well to obtain Std 8.
- Take 100 μl of Std 8, add 200 μl of Diluent, mix well, and obtain Std 7.
- Using the same method for tripling dilutions, you can obtain Std 6 - Std 1.

Standard	Dilution Method	IgG Standard Concentration, 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

▲ Take the IgG Standard and dissolve it in ddH2O, then aliquot and store at temperatures between -30 ~ -15℃ to avoid repeated freezing and thawing.

07-2/Sample Preparation

Samples should be diluted with Diluent or freshly prepared buffer containing 0.5% BSA at pH 7.0.

07-3/Reaction System

1. Sample Adding

The reaction volume for 96/384 shallow well plate is 20 μl. Add samples according to the experimental groups and reaction systems in the table below.

	Standard/Sample	Negative Control	Eu Control	Buffer Control
IgG Standard/Sample	10 μl	–	–	–
Anti-hFc-Eu	5 μl	5 μl	5 μl	–
Anti-hFab-A2	5 μl	5 μl	–	–
Diluent	–	10 μl	10 μl	10 μl
Detection buffer	–	–	5 μl	10 μl

2. The order of adding reagents is as follows:

- Add 10 μl of IgG standard/samples to the 96/384 shallow well plates.
- Add 5 μl of Anti-hFab-A2, gently mix twice inside the well using a pipette.
- Add 5 μl of Anti-hFc-Eu, gently mix twice inside the well with a pipette. (Mix Anti-hFab-A2 and Anti-hFc-Eu in a 1:1 volume ratio, add 10 μl to the reaction system, and gently mix twice inside the well with a pipette.)

Incubate at room temperature or 25℃ for 2 hours, and detect using a plate reader (equipped with an HTRF module), with excitation at 320 nm and emission at two wavelengths (665 nm and 620 nm).

08/Data Processing

Divide the fluorescence value at 665 nm by the fluorescence value at 620 nm to obtain the 665/620 ratio. Plot the log10 [sample concentration] on the X-axis and the 665/620 value on the Y-axis, and perform curve fitting to create the curve.

Std No.	[IgG Standard], ng/ml	665/620
Std 8	2000	4.4166
Std 7	666.67	3.8872
Std 6	222.22	3.3026
Std 5	74.07	2.1370
Std 4	24.69	0.9927
Std 3	8.23	0.4833
Std 2	2.74	0.2723
Std 1	0.91	0.1999
Negative control	–	0.1641