ELISA Reagent Kit for Human Fc Protein (Key Reagents Only)

Catalog number: D3020

Two sizes: sufficient for 20 microplates, sufficient for 100 microplates

For the quantitative determination of human Fc proteins and human IgGs in cell culture supernatant and serum

***** This assay is specific for human Fc, and does not cross react with mouse, rat, rabbit, cat and dog Fc.

* This package insert must be read in its entirety before using this product.

Revision: 05/2019

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

TABLE OF CONTENTS

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INTRODUCTION

Human Fc is the tail region of an immunoglobuin G (IgG) that interacts with cell surface receptors called Fc receptors and some proteins of the complement system. The ~230 amino acid fragment generally exists as a dimer, although under reducing conditions, it exists as a monomer. It is the basis of prolonged pharmacokinetics of antibodies and is commonly used in a fusion to extend the half-life of fusion proteins.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An affinity purified polyclonal antibody specific for human Fc is coated onto a 96-well microplate. Standards, control, and samples are pipetted into the wells and any human Fc present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Fc is added to the wells. Following an incubation and a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution is added. The intensity of the color measured is in proportion to the amount of human Fc bound in the initial step. The sample values are then read off the standard curve.

PRECAUTION

The Stop Solution provided in this kit is a solution of low pH. Wear eye, hand, face, and clothing protection when using this material.

MATERIALS PROVIDED

	DESCRIPTION	D3020 – 20 plates	D3020 – 100 plates
1	**Capturing Antibody** - a polyclonal antibody specific for human Fc protein	1 vial (500 µL)	2 vials (1250 µL/vial)
2	**HuFc Detection Antibody** - a horseradish peroxidase (HRP)-conjugated polyclonal antibody against human Fc protein	1 vial (100 µL)	1 vial (500 µL)
3	**Human Fc Protein Standard** - 10,000 ng/mL human Fc protein	1 vial (200 µL)	1 vial (1000 µL)

MATERIALS NOT PROVIDED

	DESCRIPTION
1	**96-well or 384-well ELISA Microplate**
2	**3% BSA in PBS**
3	**Wash Buffer** - PBS
4	**Assay Diluent** - PBS with 1% BSA
5	**Color Reagent** - Tetramethylbenzidine (TMB)
6	**Stop Solution** - 0.5 N Hydrochloric acid solution

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, optionally with correction wavelength set at 540 or 570 nm
- Pipettes and pipette tips
- Polypropylene test tubes
- Deionized or distilled water

STORAGE

- Do not freeze
- Store at 2-8 ℃
- Best if used within 3 months of receipt

MICROPLATE PREPARATION PROCEDURE

1. For each 96-well microplate, prepare 5 mL of PBS solution (pH 7.4) containing 20 µL of Capturing Antibody, and aliquot 50 µL per well. Incubate over night at 4ºC. Alternatively, use sodium bicarbonate buffer (100 mM, pH 9.5) in place of PBS as coating buffer.
2. Remove liquid and wash wells one time with PBS (200 µL per well)
3. Block plate with 3% BSA in PBS (200 µL per well) for at least 1 hour at room temperature
4. Remove liquid and wash wells one time with PBS (200 µL per well)
5. Coated plates can be stored foil wrapped at 4ºC for up to 2 weeks

SAMPLE PREPARATION

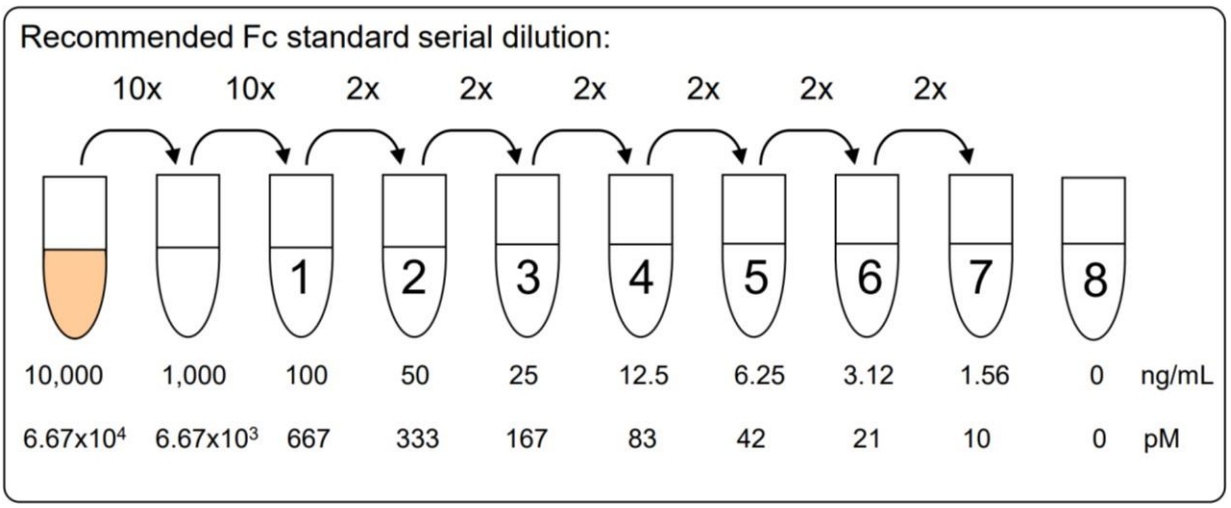
Cell culture supernatants - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20℃. Avoid repeated freeze-thaw cycles.

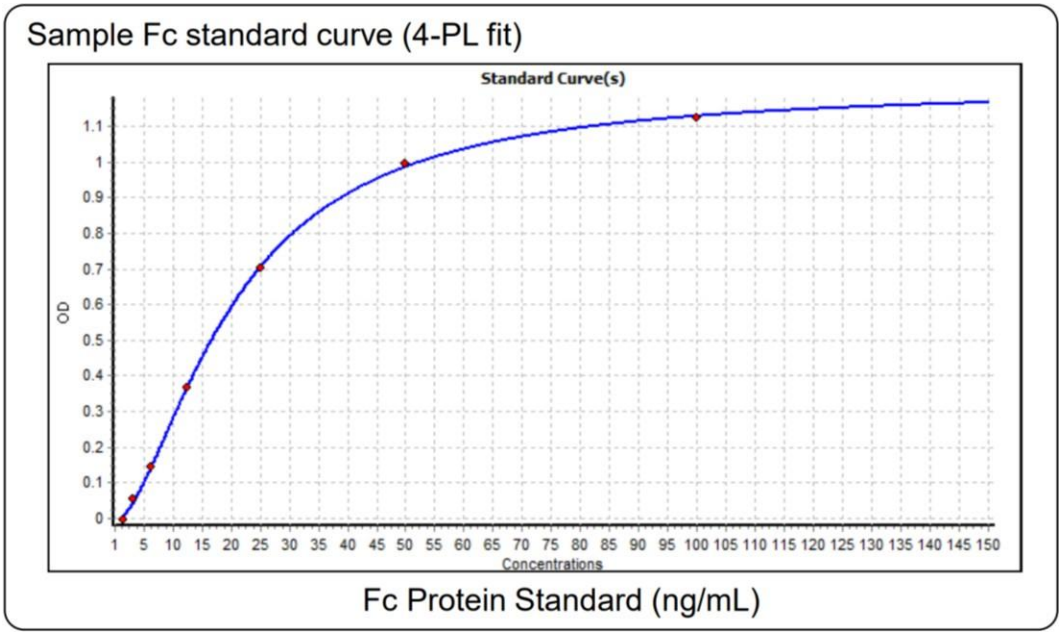
Serum - Allow samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifuging for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or store samples at ≤-20℃. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20℃. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use
2. Make appropriate dilutions of Fc Protein Standard in Assay Diluent provided
3. Make appropriate dilution of test samples in Assay Diluent (1% BSA in PBS)
4. Prepare 1x PBS as Wash Buffer
5. Prepare Detection Antibody Solution by diluting Detection Antibody 1:1000 in Assay Diluent
6. Prepare 0.5 N Hydrochloric acid as Stop Solution





Note on standard curve linearity: The linearity of standard curves is influenced by various factors including incubation time, temperature, amount of secondary antibody, and reaction substrate. With advanced data analysis software, standard curves do not need to be perfectly linear for suitable data analysis and conversion. MEDNA uses and recommends BMG LABTECH Microplate Readers and MARS Data Analysis Software (www.bmglabtech.com).

ASSAY PROCEDURE

1) Prepare reagents, standard curve serial dilution, and test samples as directed in the previous section
2) Cover the unused microplate wells with plastic seal for future use
3) Add 50 µL/well of standard dilutions and appropriately diluted samples
4) Incubate for 1 hour or longer at room temperature, or for 30 minutes in 37°C
5) Remove liquid from wells
6) Wash each well 3 times with 200 µL/well of **1x Wash Buffer**
7) Add 50 µL/well of **Human Fc Detection Antibody**
8) Incubate for 30 minutes or longer at room temperature, or in 37°C
9) Remove liquid from wells
10) Wash each well 3 times with 200 µL/well of **1x Wash Buffer**
11) Add 50 µL/well of **Color Reagent**
12) Incubate at room temperature until color develops (2-15 minutes)
13) Read absorbance on a microplate reader at 650 nm (optional)
14) Add 50 µL/well of **Stop Solution**

    (CAUTION: STOP SOLUTION IS ACIDIC; WEAR PROPER PROTECTION)
15) Read absorbance on a microplate reader at 450 nm immediately
16) Use 540 nm or 570 nm for wavelength correction (optional)

MATERIAL SAFETY DATA
Hazard information is provided for compliance with both the UK Chemicals (Hazard Information and Packaging) (CHIP) Regulations and the US Hazard Communication Standard (HCS)

Hazards Identification:
STOP SOLUTION (0.1 N hydrochloric acid) is a diluted hydrochloric acid. Hydrochloric acid is a hazardous material. CAS NO.7647-01-0. Molecular Weight: 36.46 Chemical Formula: HCl

Emergency Overview
POISON! DANGER! CORROSIVE. LIQUID AND MIST CAUSE SEVERE BURNS TO ALL BODY TISSUE. MAY BE FATAL IF SWALLOWED OR INHALED. INHALATION MAY CAUSE LUNG DAMAGE.

SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 3 - Severe (Poison)
Flammability Rating: 0 - None
Reactivity Rating: 2 - Moderate
Contact Rating: 4 - Extreme (Corrosive)
Lab Protective Equip: GOGGLES & SHIELD; LAB COAT & APRON; VENT HOOD; PROPER GLOVES
Storage Color Code: White (Corrosive)

Personal Protection
Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, and gloves. Avoid contact of material with skin or eyes. Ensure access to a safety shower and eye-wash.

First Aid Measures
Inhalation: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.
Ingestion: DO NOT INDUCE VOMITING! Give large quantities of water or milk if available. Never give anything by mouth to an unconscious person. Get medical attention immediately.
Skin Contact: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.
Eye Contact: Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

UNIT CONVERSION

Molarity of Standards:

The Fc standard serial dilution is equivalent to:
6.67×10^4, 6.67×10^3, 667, 333, 167, 83, 42, 21, 10, 0 pM

Conversion of (X) ng/mL to (Y) nM:

$$\frac{\text{X ng/mL}}{\text{MW (g/mol)}} = \frac{\text{X ng}/10^{-3}\text{ L}}{\text{MW (ng/nmol)}} = \text{Y nM}$$

Example:

Sample concentration = 120 ng/mL
MW Sample = 150 kD

$$\frac{120\text{ ng/mL}}{150{,}000\text{ (g/mol)}} = \frac{120\text{ ng}/10^{-3}\text{ L}}{150{,}000\text{ (ng/nmol)}} = 0.8\text{ nM}$$

If molecular weight of sample differs from Fc standard (MW 150kD) apply the following equation to the reading concentration to obtain the actual concentration:

$$\frac{\text{[MW Sample]}}{\text{[MW Fc Protein]}} \times \text{Sample reading Concentration (ng/mL)} = \text{Sample Concentration after MW adjustment (ng/mL)}$$

Example:

MW Fc Protein = 150 kD
MW Sample = 75 kD
Sample calculated concentration = 40 ng/mL

$$\frac{\text{[75,000 g/mol]}}{\text{[150,000 g/mol]}} \times 40\text{ ng/mL} = 20\text{ ng/mL}$$

If the molecular weight of the sample is smaller than that of the Fc standard, then the actual concentration of the sample is lower than the sample reading concentration.