MEDNA

96-Well Mouse Fc ELISA Kit

Catalog number: D2007

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

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

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

Revision: 03/2020

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INTRODUCTION

Mouse 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 reduced condition, it exists as a monomer. It is the basis of prolonged pharmacokinetics of antibodies and is commonly used as 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 mouse Fc has been coated onto a 96-well microplate. Standards, control, and samples are pipetted into the wells and any mouse Fc present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse 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 mouse 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	Parts
1	**Pre-coated Microplate** - 96 well polystyrene microplates coated with polyclonal antibody specific for mouse Fc protein	2 plates
2	**MuFc Detection Antibody (100X)** - a horseradish peroxidase (HRP)-conjugated polyclonal antibody against mouse Fc protein	1 vial (500 µL)
3	**Mouse Fc Protein Standard** - 10,000 ng/mL mouse Fc protein	1 vial (50 µL)
4	**Conjugate Buffer** - buffer with preservatives and stabilizer	1 bottle (26 mL)
5	**Assay Diluent** - PB buffer with BSA	1 bottle (60 mL)
6	**Wash Buffer, 50x Concentrate** - PBS buffer with detergent	1 bottle (12 mL)
7	**Color Reagent** - tetramethylbenzidine (TMB)	1 bottle (12 mL)
8	**Stop Solution** - 0.5 N hydrochloric acid solution	1 bottle (12 mL)

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 2-8ºC
- Best used within 3 month of receipt

SAMPLE PREPARATION

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

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

REAGENT PREPARATION

1. Bring all reagents to room temperature before use

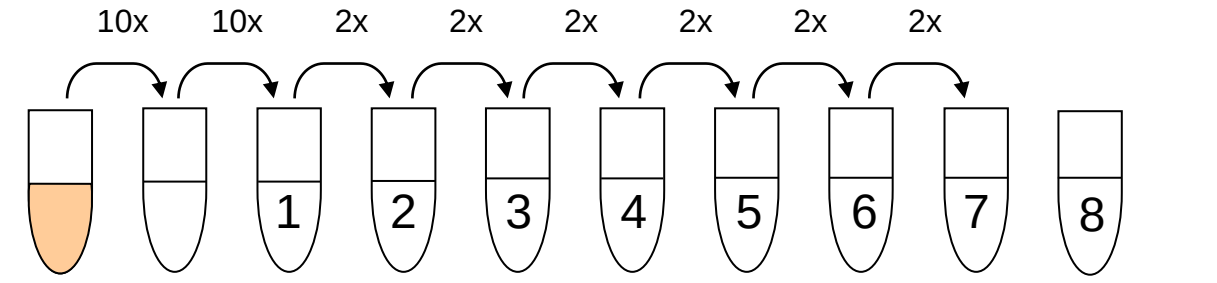
2. Make appropriate dilutions of **Fc Protein Standard** in **Assay Diluent** provided

3. Make appropriate dilution of test samples in **Assay Diluent** provided

4. Prepare **1x Wash Buffer** by diluting appropriate amount of **50x Wash Buffer Concentrate** in deionized or distilled water

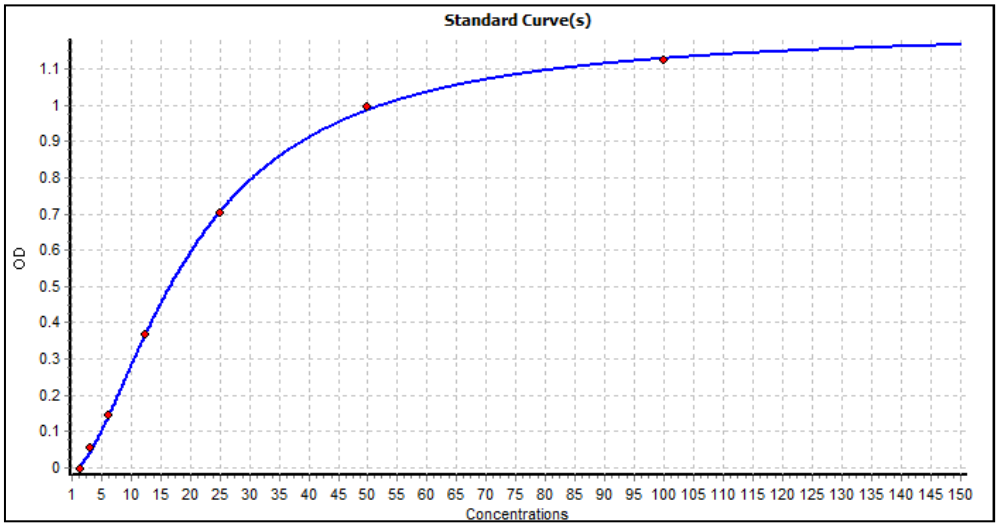
5. To prepare Mouse Fc Detection Antibody by diluting appropriate of **Mouse Fc Detection Antibody (100x)** in Conjugate Buffer

Recommended Fc standard serial dilution:



10,000	1,000	100	50	25	12.5	6.25	3.12	1.56	0	ng/mL
$6.67x10^4$	$6.67x10^3$	667	333	167	83	42	21	10	0	pM

Sample Fc standard curve (4-PL fit)



Fc Protein Standard (ng/mL)

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

- Prepare reagents, standard curve serial dilution, and test samples as directed in the previous section
- Cover the unused microplate wells with plastic seal for future use
- Add 50 µL/well of standard dilutions and appropriately diluted samples
- Incubate for 1 hour or longer at room temperature, or for 30 minutes in 37^{o}C
- Remove liquid from wells
- Wash each well 3 times with 200 µL/well of **1x Wash Buffer**
- Add 50 µL/well of **Mouse Fc Detection Antibody**
- Incubate for 30 minutes or longer at room temperature, or in 37^{o}C
- Remove liquid from wells
- Wash each well 3 times with 200 µL/well of **1x Wash Buffer**
- Add 50 µL/well of **Color Reagent**
- Incubate at room temperature until color develops (2-15 minutes)
- Read absorbance on a microplate reader at 650 nm (optional)
- Add 50 µL/well of **Stop Solution**
  (CAUTION: STOP SOLUTION IS ACIDIC; WEAR PROPER PROTECTION)
- Read absorbance on a microplate reader at 450 nm immediately
- 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.