

For the quantitative determination of human Calprotectin concentrations in cell culture supernatants, serum, and plasma.

INTRODUCTION

Calprotectin is a calcium- and zinc-binding protein found primarily in the cytoplasm of neutrophils, but also in monocytes and macrophages. Calprotectin is highly resistant to enzymatic degradation, and it is used as a non-invasive marker of intestinal inflammation, especially in conditions such as inflammatory bowel disease (IBD). Fecal calprotectin levels are well correlated with intestinal mucosal neutrophil infiltration and can be used to differentiate between organic and non-organic intestinal diseases with high sensitivity and specificity. Elevated levels of calprotectin can also be found in other inflammatory diseases such as rheumatoid arthritis.

The Tribio™ Fast Human Calprotectin ELISA is designed to quantitatively detect Human Calprotectin levels in different tissues including skin, muscle, neural, serum, and other biological samples. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 3 hours, not needing 4-5 hours (Fig. 1). The detection range is from 6 to 400 pg/mL. The levels of human Calprotectin samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human Calprotectin protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human Calprotectin was pre-coated onto a microplate. Standards or samples are pipetted into the wells and then incubated with biotin-conjugated detection antibody specific for human Calprotectin. After aspirating the plate without wash, add Streptavidin-HRP and incubate for an indicated time. Following a wash to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color development. The color intensity is in proportion to the amount of Calprotectin bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

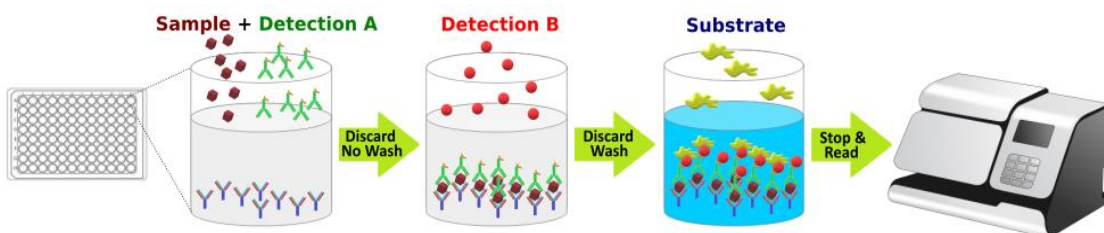


Fig.1: Simple ELISA procedure

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human Calprotectin Microplate	TBS3262A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human Calprotectin.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human Calprotectin Standard	TBS3262B	30 µl of Recombinant human Calprotectin protein (40 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3262C	2.1 ml of Biotin-Human Calprotectin antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3262D	300 µl of Streptavidin-HRP.	
Assay Diluent	TBS3262E	25 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*)

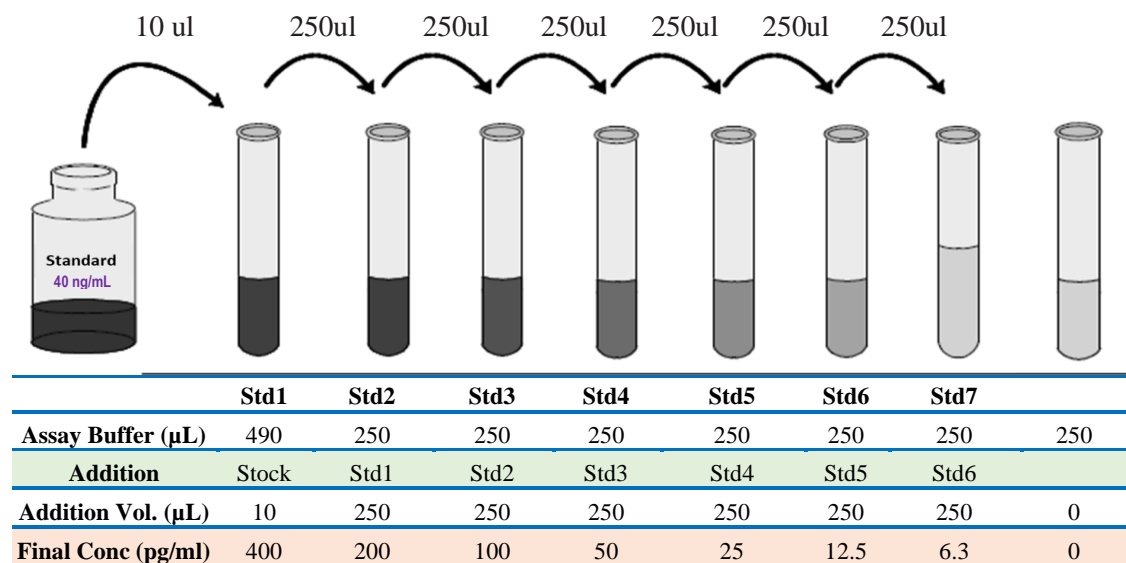
Detection B working solution preparation: Add 240 μ L of **Detection B** streptavidin-HRP to 12 mL Assay Diluent to prepare Detection B working solution.

Human Calprotectin Standard Preparation:

Label test tubes as #1 through #8. Pipet 490 μ L of 1x Assay Diluent into tube #1, and 250 μ L into tubes #2 to #7 as diagram below (Fig.2).

1. Add 10 μ L of the Human Calprotectin Standard stock solution (40ng/mL) by dilution of 100X to tube #1 (400pg/mL), and mix.
2. Make 2x serial dilutions of the standard using the 400pg/mL standard solution (tube #1) from tube #2 through #7 with sequential transfer of 250 μ L to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 400, 200, 100, 50, 25, 12.5, and 6.3 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human Calprotectin standard preparation



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 μ L of standard, sample, or control per well.
2. Add 20 μ L of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 μ L of **Detection B** to each well. Incubate at **RT for 1 hour**.
5. Aspirate each well, and wash for 3 times by filling each well with 300 μ L Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 μ L of **TMB Substrate** to each well. Incubate **at RT for 10-20 min** (*Protect from light*). The color becomes blue.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Calprotectin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve ($R^2=0.9995$) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

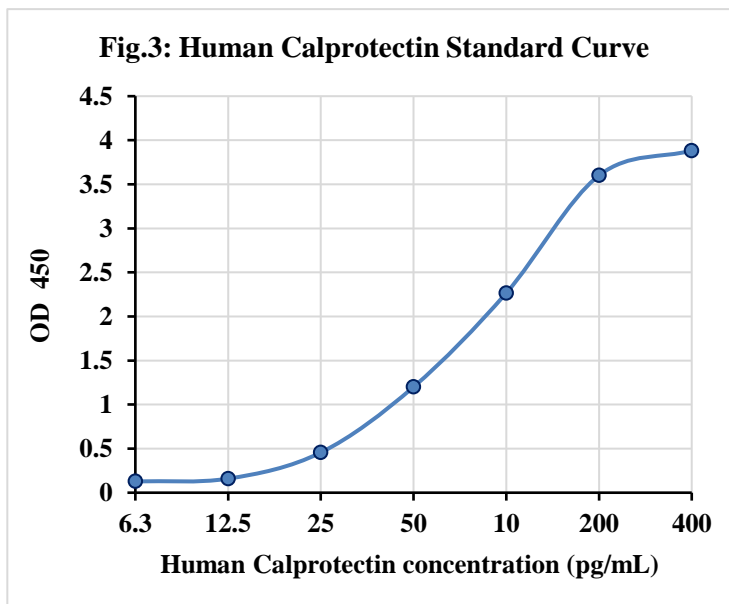
The minimum detectable dose (MOD) of human Calprotectin is typically 10 pg/ml. The Intra-assay CV is 3.79% the Inter-assay CV is <10%.

SPECIFICITY

This assay recognizes natural and recombinant human Calprotectin.

RELATIVE PRODUCTS

- Human CEA ELISA (TBS3210)
- Human AFP ELISA (TBS3212)
- Human HE4 ELISA (TBS3213)
- Human IL-1β ELISA (TBS3219)
- Human IL-2 ELISA (TBS3220)
- Human IL-4 ELISA (TBS3221)
- Human IL-6 ELISA (TBS3223)
- Human IL-7 ELISA (TBS3224)
- Human IL-8 ELISA (TBS3225)
- Human IL-10 ELISA (TBS3226)
- Human IL-13 ELISA (TBS3227)
- Human IL-17 ELISA (TBS3228)
- Human IL-22 ELISA (TBS3229)
- Human IFN-gamma ELISA (TBS3230)
- Human TGF-β1 ELISA (TBS3232)
- Human GM-CSF ELISA (TBS3233)
- Human MIP-1α ELISA (TBS3234)
- Human TNF-α ELISA (TBS3235)
- Human IL-18 ELISA (TBS3239)



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