Fast Human IL-12 p70 ELISA kit

For the quantitation of human IL-12 P70 concentrations in cell culture supernatants, serum, and plasma.

INTRODUCTION

Interleukin 12 (IL-12) is an anti-inflammatory cytokine made up of a 40 kDa (p40) subunit and a 35 kDa (p35) subunit, also known as natural killer cell stimulatory factor (NKSF) or cytotoxic lymphocyte maturation factor (CLMF). The IL-12 and IL-23 (another heterodimeric cytokine) share the p40 subunit. IL-12 inhibits the synthesis of numbers of cytokines, including IFN-gamma, IL-2, IL-3, TNF and GM-CSF produced by activated macrophages and by helper T-cells.

The Fast Human IL-12 p70 ELISA is a solid phase ELISA designed to measure human IL-12 p70 levels in cell culture supernatants, serum, and plasma. 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 1 hour, with no need for 5-6 hours (Fig. 1). The detection range is from 8 to 2000 pg/mL. The levels of human IL-12 p70 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 IL-12 protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). A monoclonal antibody specific for human IL-12 was pre-coated onto a microplate. Standards or samples and detection antibody are pipetted into the wells, then, concurrently incubated to form a sandwich complex in one-step. Simply aspirate each well without washing, directly add Streptavidin-HRP into the complex. Following a wash, an ultra-sensitive TMB substrate solution is added to the wells for color development. The color intensity is in proportion to the amount of IL-12 bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

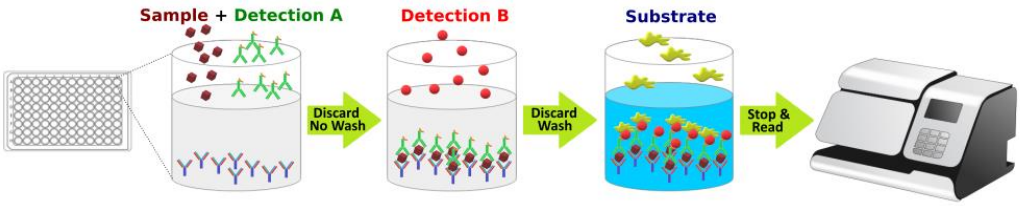


Fig.1 Simple ELISA procedure.

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human IL-12 Microplate	TBS3204A	96-well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human IL-12 p70.	The unused wells can be stored in the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Human IL-12 Standard	TBS3204B	Recombinant human IL-12 protein (Lyophilized). Reconstitute to 0.5 mL Assay Buffer.	Store at 2-8 °C for 6 months. After reconstituting in 0.5mL Assay Buffer, it can be stored at -20 °C for up to 1 month.
Detection A	TBS3204C	2.1 mL of human IL-12 antibody.	May be stored for up to 6 months at 2-8 °C.
Detection B	TBS3204D	0.25 mL of Streptavidin-HRP (1:50 Dilution)	
Assay Diluent	TBS3204E	25 mL of a buffered protein base with preservatives.	
10x 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 ℃. 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. It is crucially important for good performance.

Reagent preparation:

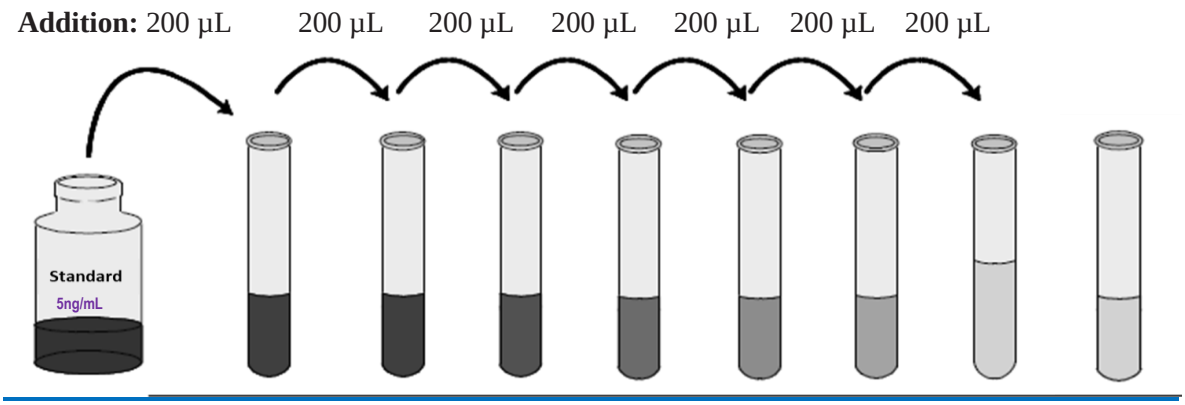
Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

Detection B working solution: Dilute Detection B Stock Solution with 1:50 ratio. Add 200 µL Detection B Stock Solution to 10 mL Assay Buffer.

Human IL-12 p70 Standard Preparation:

1. **Reconstitute Standard:** Add 0.5mL Assay Buffer to lyophilized standard vial to make 5ng/mL standard stock solution.
2. Label test tubes as #1 through #8. Pipet 300 µL Assay Buffer into tubes #1 to #8 **as diagram below.**
3. Add 200 µL of the Human IL-12 p70 Standard stock solution (5 ng/mL) by dilution of 25X to tube #1 and mix.
4. Make 2.5x serial dilutions of the standard using the 2000pg/mL standard solution in tube#1 from tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 2000, 800, 320, 128, 51.2, 20.48 and 8.192 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human IL-12 p70 standard preparation



Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	300	300	300	300	300	300	300	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	200	200	200	200	200	200	200	0
Final Conc (pg/ml)	2000	800	320	128	51.2	20.48	8.192	0

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 µL of standard, sample, or control per well.
2. Add 20 µL 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. Invert the plate and blot it against clean paper towels (***no wash***).
4. Add 100 µL 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 µL 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 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue. If the color is light, the incubation time can be longer.
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 5 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 IL-12 p70 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=0.998) is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

SENSITIVITY

The minimum detectable dose (MOD) of human IL-12 p70 is typically 6.5 pg/ml.

The Intra-CV is < 10% and the Inter-CV is < 12%.

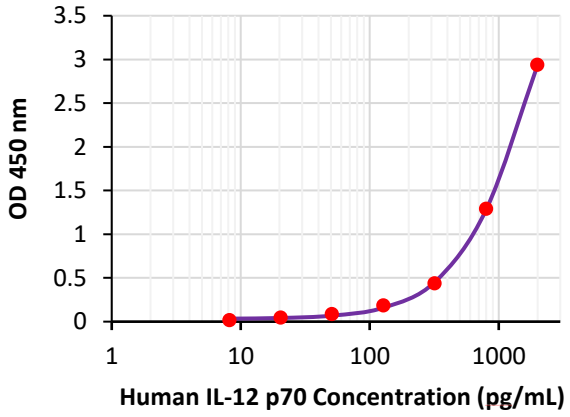
SPECIFICITY

This assay recognizes natural and recombinant human IL-12 P70.
No-cross reactivity: Human IL-12 p35; Human IL-12/IL-23 p40; Human IL-23; Mouse IL-12 p70.

RELATIVE PRODUCTS

Human IL-1ß 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-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Human TNF-α ELISA (TBS3235)
Human IL-18 ELISA (TBS3239)

Fig.3 Human IL-12 p70 Standard Curve



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