

1 PRINCIPLE OF THE ASSAY

The Immunotech IL-8 enzyme immunoassays (IM2237, IM2238) are Research Use Only products, intended for quantification of human interleukin 8 (IL-8) in plasma, serum or culture supernatants. This ELISA is a two immunological step sandwich type assay. In the first step the IL-8 is captured by a monoclonal antibody bound to the wells of a microtiter plate. In the second step a biotinylated monoclonal antibody is added together with streptavidin-peroxidase conjugate. The biotinylated antibody binds to the solid phase antibody-antigen complex and, in turn, binds the conjugate. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromogenic substrate. The intensity of the coloration is proportional to the IL-8 concentration in the sample or standard.

2 REAGENTS PROVIDED

The kit should not be used beyond the expiration date on the kit label.

All reagents in the unopened kit are stable at 2-8°C until the kit expiration date.

Storage conditions for opened and reconstituted reagents are indicated in the following table.

Reagents	Description		Preparation or reconstitution	Storage of opened or reconstituted reagents
	kit IM2237 (96 wells)	kit IM2238 (5 x 96 wells)		
Plate 96 wells	1	5	ready-to-use	store at 2-8°C in self-lock bag until expiration date
Standard lyophilized	1 vial	2 vials	add the volume of distilled water stated on the vial label	1 week at 2-8°C (until expiration date, aliquoted at -18°C)
Biotinylated monoclonal antibody	1 vial, 6 mL	5 vials, 6 mL	ready-to-use	store at 2-8°C until expiration date
Diluent	1 vial, 25 mL	2 vials, 25 mL	ready-to-use	store at 2-8°C until expiration date
Streptavidin-HRP conjugate	1 vial, 12 mL	5 vials, 12 mL	ready-to-use	store at 2-8°C until expiration date
Wash solution (20 x)	1 vial, 50 mL	3 vials, 50 mL	dilute 50 mL in 950 mL of distilled water	1 month at 2-8°C, until expiration date at -18°C
Substrate	1 vial, 12 mL	5 vials, 12 mL	ready-to-use	store at 2-8°C until expiration date
Stop solution sulfuric acid *	1 vial, 6 mL	5 vials, 6 mL	ready-to-use	store at 2-8°C until expiration date

* 2N sulfuric acid is considered as irritant at this concentration. Read precautions (§ 4).

3 SUPPLIES REQUIRED BUT NOT PROVIDED

Standard laboratory equipment is required, including precision adjustable micropipets, microtiter plate shaker (shaking vigorously without splash-over, i. e. 350 rpm), microtiter plate reader (450 nm filter), microplate washer (optional).

4 PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The stop solution is classified as irritant for eyes and skin. In case of contact rinse immediately with plenty of water and seek medical advice. Prevent contact by using rubber gloves and wearing lab coat.

5 SAMPLE COLLECTION AND STORAGE

Sample processing is critical for cytokine assays. Avoid any stimulation of the cells while performing the procedure. All sampling material must be pyrogen-free. We recommend the use of EDTA plasma. Serum, plasma or culture supernatant processed as recommended below, may be assayed with this kit.

Cell culture supernatant: remove particulate by centrifugation.

Serum: allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum rapidly and carefully from clot.

Plasma: collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge for 15 minutes at 1000 g within 1 hour of collection.

Other samples: may be assayed using appropriate standard series. To prepare the standard series, use a diluent resembling the sample, containing no IL-8 but at least 2g/L of protein.

Collection and storage: most cytokines are labile molecules in biological fluids. Assay the samples immediately or aliquot in plastic tubes and store at <-18°C (maximum of 2 months) or preferably at <-70°C for longer periods. Avoid repeated freeze-thaw cycles.

Dilution: dilute samples containing IL-8 concentrations above 2000 pg/mL in the same diluent as the one used to dilute the standard.

6 ASSAY PROCEDURE

Preliminary notes:

- Let the components of the kit equilibrate 30 minutes at room temperature before use.
- Do not mix reagents from different lots.
- Wait 10 minutes after solubilization of lyophilized components and mix gently to avoid foaming. Do not vortex.
- It is recommended that assays be performed in duplicate.
- Standards and samples must be assayed at the same time.
- Pipeting time for substrate and stop solution should not exceed 3 minutes. Dispensing must be done in the same order. Avoid exposing the substrate to direct sunlight.

Reagent preparation:

- Dilute the wash solution (20 x) with 950 mL of distilled water.
- Reconstitute the lyophilized standard with the volume of distilled water stated on the vial label. This will result in a 20 ng/mL IL-8 solution, store at 2-8 °C for up to one week, or freeze according to recommendations for longer periods (see §5 collection and storage).
- From the 20 ng/mL standard solution and the appropriate diluent, prepare a fresh dilution series in plastic tubes prior to each assay as indicated below. This dilution series cannot be stored.

Standard concentration	IL-8	Diluent
2000 pg/mL	50 µL of 20 ng/mL standard	450 µL
500 pg/mL	100 µL of 2000 pg/mL standard	300 µL
125 pg/mL	100 µL of 500 pg/mL standard	300 µL
31.2 pg/mL	100 µL of 125 pg/mL standard	300 µL
0 pg/mL	-	300 µL

Protocol:

Step 1	Step 2	Step 3
Add 50 µL of standard or sample per well	Add 50 µL of biotinylated antibody and 100 µL of streptavidin-HRP conjugate	Add 100 µL of substrate
Incubate 2 hr at 18-25°C while shaking	Incubate 30 min at 18-25°C while shaking	Incubate 20 min at 18-25°C while shaking
Wash the wells*	Wash the wells	Add 50 µL of stop solution
		Read absorbance at 450 nm

** Plate washing:*

This step is essential to obtain the expected kit performance. After washing, wells must not dry prior to the addition of the next reagent.

Using a microtiter plate washer

Select a three cycle plate washing program that meets the following cycle criteria :

Fluid in wells must be completely aspirated

Wells must be filled to the rim with the wash solution

Wash solution must be injected rapidly (typically one second to fill one well)

After the three cycles, wash solution must be completely aspirated.

Manual procedure

Repeat at least four cycles as follows:

Turn plate upside-down and shake vigorously over the sink.

Fill wells with wash solution, the solution may run over the rim of the wells

Turn plate upside-down and shake vigorously over the sink, and firmly tap the inverted microtiter plate onto a clean absorbent paper.

7 RESULTS

The sample results are calculated by interpolation from a standard curve that is performed in the same assay as that of the sample. Draw the curve, plotting on the horizontal axis the IL-8 concentration of the standards and on the vertical axis the corresponding absorbance. Locate the absorbance for each sample on the vertical axis and read off the corresponding IL-8 concentration on the horizontal axis.

Using a computer, we recommend the use of a curve fit equation $Y = A + Bx + Cx^2$ (quadratic mode).

Example of standard curve (not to be used for actual calculation)

IL-8 (pg/mL)	Absorbance (mAbs 450 nm)	CV %
2000	1843	2.5
500	459	1.2
125	127	8.9
31.3	53	0
0	28	2.5

8 PERFORMANCE CHARACTERISTICS

Sensitivity:

Defined as the lowest IL-8 concentration significantly different from the zero standard with a probability of 95%, sensitivity is 8 pg/mL.

Specificity:

The assay measures human, natural or recombinant IL-8. No cross-reactivity or interference with other cytokines or cytokine receptors is known.

Calibration:

The standard is calibrated by reference to the WHO IL-8 (89/520) standard.

Precision:

Intra-assay precision was determined by assaying sera or plasma 10 times. CVs ranged between 2.3 and 5.5%.

Inter-assay precision was determined by assaying sera or plasma 10 times in independent assays. CVs ranged between 7.6 and 10.1%.

Recovery:

IL-8 was added at different concentrations using 3 different plasma samples. The observed recovery was between 82 and 88%.

Dilution tests:

Three different plasma samples containing IL-8 were diluted to 1:16 using the kit diluent. The observed recovery was between 93 and 119%.

Drift:

No drift is observed when time for distribution of samples does not exceed 60 min.