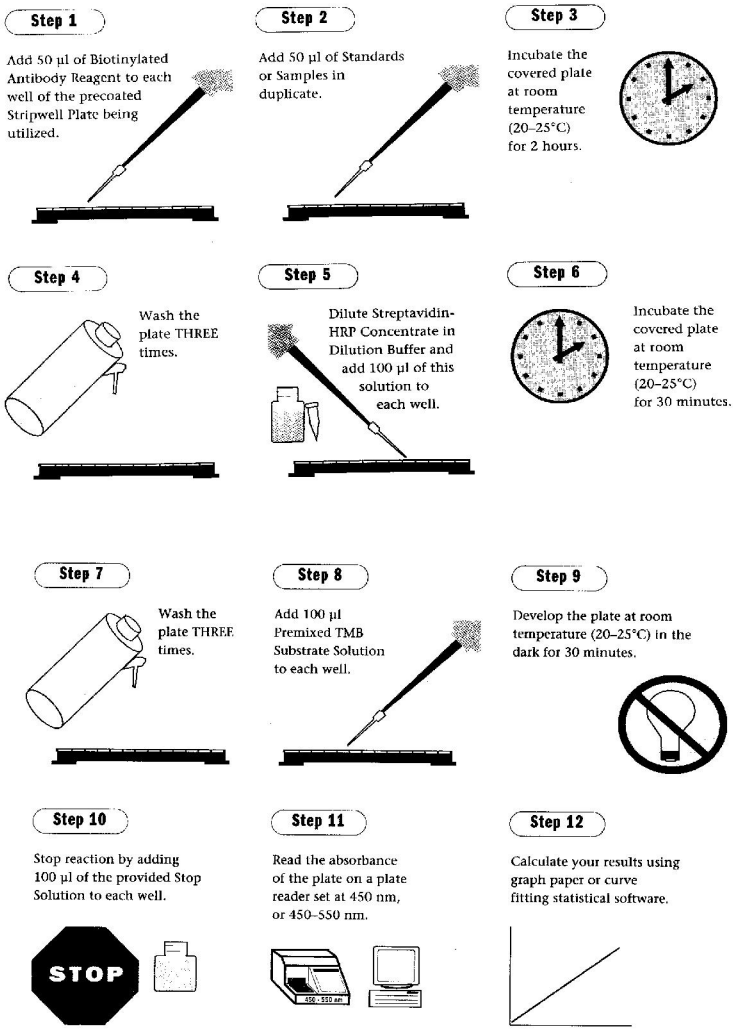
DRG ® hIL-6 (EIA-1200)

Revised 13 Aug. 2009 (Vers. 1.1) **RUO**

Introduction

The Human Interleukin-6 (IL-6) ELISA is an enzyme-linked immunosorbent assay for measurement of human IL-6 serum, plasma, urine and culture supernatants. This kit is intended for Research Use Only.



Materials

Materials Provided

- Anti-Human IL-6 Precoated Strip Well Plate- 1
- Lyophilized E. *coli*-derived Recombinant Human IL-6 Standard- 2 vials
- Standard Diluent-25 ml, contains 0.1% sodium azide
- Biotinylated Antibody Reagent- 8 ml, contains 0.1% sodium azide.
- 30X Wash Buffer, 50 ml.
- Streptavidin-HRP Concentrate- 0.075 ml

- Streptavidin-HRP Dilution Buffer- 14 ml.
- TMB Substrate Solution- 13 ml
- Stop Solution- 13 ml, contains 0.16 M sulfuric acid
- Adhesive Plate Covers- 6

Additional Materials Required

1. Precision pipettors with disposable plastic tips to deliver 5 to 1000 µL.
2. Plastic pipetts to deliver 5 to 15 ml.
3. A glass or plastic two-liter container to prepare Wash Buffer.
4. A squirt wash bottle, or an automated 96-Well plate washer.
5. 1.5 ml polypropylene or polyethylene tubes to prepare standards. **Do not use polystyrene, polycarbonate or glass tubes.**
6. Disposable reagent reservoirs.
7. 15 ml plastic tube to prepare Streptavidin-HRP Solution.
8. A standard ELISA reader for measuring absorbance at 450 nm and 550nm. If a 550 nm filter is not available, the absorbance can be measured at 450nm only. Refer to the instruction manual supplied with the instrument being used.
9. Graph paper or a computerized curve-fitting statistical software package.

Precautions

All samples and reagents must be at room temperature (20-25^{O}C) before use in the assay.

- Review these instructions carefully and verify al components against the contents list before beginning.
- Do not use a water bath to thaw samples. Thaw at room temperature.
- When preparing standard curve and sample dilution in your culture medium, use the same medium you used to culture the cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture the cells, then RPMI with 10% FCS to dilute the standard and samples. DO NOT use RPMI without serum supplement.
- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- Use fresh disposable pipet tips for each transfer to avoid cross contamination.
- Use a new adhesive plate cover for each incubation step.
- Do not mix reagents from different kit lots.
- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.
- Avoid microbial contamination of reagents.
- Avoid exposure of reagents to excessive heat or light during storage and incubation.
- Discard unused ELISA components after completion of the assay.
- Do not use glass pipettes to measure TMB Substrate Solution. Take care not to contaminate the solution. If the solution is BLUE before use, DO NOT USE IT.
- Individual components of this assay kit may contain antibiotics and preservatives. Gloves should be worn while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some components of this kit contain sodium azide. Please dispose of reagents according to local regulations.

Additional Precautions for the 5-Plate Kit

- Dispense only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.
- Use only one bottle of the TMB Substrate Solution per 96-well plate. Do not combine leftover substrate with that reserved for other plates.
- Equilibrate to room temperature only the reagent volumes required for the number of plates being used.
- Use only one vial of Standard per 96-well plate.

Sample Preparation

- Serum, EDTA, heparin and sodium citrate plasma, and culture supernatants may be tested in this ELISA.
- 50 µl of serum, plasma, urine or culture supernatant are required.
- Store samples to be assayed within 24 hours at 2-8ºC. For long-term storage, aliquot and freeze samples at -70° C.
- Avoid repeated freeze-thaw cycles when storing samples.
- Test samples and standards must be assayed in duplicate each time the ELISA is preformed.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use a heated water bath to thaw or warm samples.
- Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any question about the integrity of a sample. Make a note on the template and interpret results with caution.
- If the human IL-6 concentration possibly exceeds the highest point of the standard curve (i.e., 400 pg/ml), prepare one or more 10-fold dilutions of the test sample. When testing culture supernatants, prepare the serial dilutions using your culture medium. When testing serum, plasma or urine prepare the serial dilutions using the Standard Diluent provided. For example, a 10-fold dilution is prepared by adding 0.05 ml (50µl) of the test sample to 0.45 ml (450µl) of appropriate diluent. Mix-thoroughly between dilutions before assaying.

Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout these instructions.
Note: When using the 5-plate kit, only one Standard per plate is supplied. Therefore, partial plates cannot be used.

Wash Buffer

- Label a clean glass or plastic 2 liter container "Wash Buffer". The 30X Wash Buffer may have a cloudy appearance.
- If using the 5-plate kit, add 30 ml Wash Buffer to 870 ml of water for each plate used, otherwise, add the entire contents of the30X Wash Buffer (50ml) bottle to the two-liter container and dilute to a final volume of 1.5 liters with ultrapure water. Mix thoroughly.

(PP) When using partial plates, store the reconstituted Wash Buffer at 2-8° C.

Note: Wash Buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

Standards:

- **(PP)** Reconstitute and use one vial of the lyophilized Standard per partial plate.
- Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.

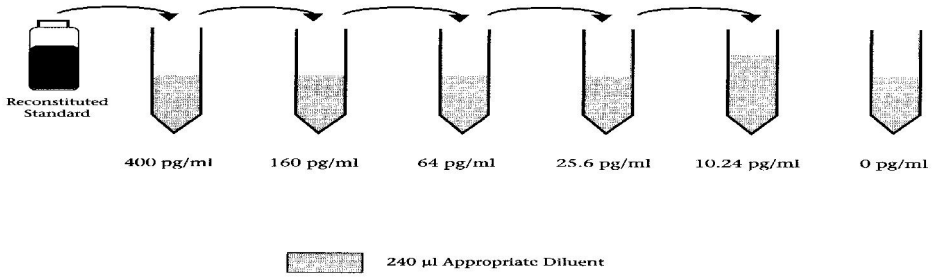
- When testing **culture supernatant samples**, reconstitute standard in ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will take approximately 1 minute to dissolve. Mix by gently inverting the vial. Use the sample culture medium to prepare Standard Curve dilutions.

- When testing **serum, plasma, or urine samples,** reconstitute standard with ultrapure water. Reconstitution volume is stated on the vial label. The standard will dissolve in approximately 1 minute. Mix gently inverting vial. Use the Standard Diluent provided to prepare standard curve serial dilutions.

- When testing **serum, plasma, or urine and cell culture supernatant samples on the same plate,** validate the media to establish if the same standard curve can be used for the different sample types. Prepare a standard curve (including a zero/blank) using culture medium to reconstitute and dilute the standard. Use the medium containing serum or other protein to maximize stability of the human IL-6. Perform this curve in parallel with a standard curve prepared with Standard Diluent. If the OD values of the two curves are within 10% of the mean for both curves, the assay can be performed with Standard Diluent, whether you are testing culture supernatant, urine, plasma, or serum samples.

- Label 6 tubes, one of each standard curve point: 400 pg/ml, 160 pg/ml, 64 pg/ml, 25.6 pg/ml, 10.24 pg/ml and 0 pg/ml. Then prepare 1:2.5 serial dilutions for the standard curve as follows:
- Pipette 240 µl of appropriate diluent (see steps c and d above) into each tube.
- Pipette 160 µl of the reconstituted standard into the first tube, 400 pg/ml and mix.
- Pipette 160 µl of this dilution into the second tube labeled 160 pg/ml and mix.
- Repeat serial dilutions (using 160 µl) three more times, to complete the standard curve points. These concentrations, 400 pg/ml, 160 pg/ml, 64 pg/ml, 25.6 pg/ml, 10.24 pg/ml and 0 pg/ml are the standard curve.

Serial Dilutions of 160 µl



Assay Procedure

Sample and Biotinylated Antibody Reagent Incubation:

- **(PP)** Determine the number of strips required. Leave these strips in the plate frame. Place the remaining unused strips back in the foil pouch with the desiccant. Make sure the foil pouch is sealed tightly. After completing the assay, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.

- Use the Data Template provided to record the locations of the zero standard (blank or negative control), IL-6 Standards and test samples. Perform five Standards and one blank in duplicate with each series of unknown samples.

- If using a multichannel pipettor, **use a new reagent reservoir** to add the Biotinylated Antibody Reagent. Remove from the vial only the amount required for the number of strips being used. Take care not to touch the samples in wells with the pipette tip when adding the Biotinylated Antibody Reagent.

- Add 50 µl of the Biotinylated Antibody Reagent to each well.

- Add 50 µl of reconstituted standards or test samples in duplicate to each well. Mix well by gently tapping the plate several times.

- **Note:** If the human IL-6 **concentrations in any test sample possibly exceeds the highest point on the standard curve**, 400 pg/ml, see section Sample Preparation.

- Add 50 µl of Standard Diluent to all wells that do not contain standards or samples.

- Carefully cover the plate with an adhesive plate cover, making sure all edges and strips are sealed tightly. Do this by running your thumb over the edges and down each strip. Incubate for two (2) hours at room temperature, 20-25^{O}C.

- Carefully remove the adhesive plate cover. Wash the plate **THREE** times with Wash Buffer, using the procedure described below.

Wash the Plate:

- Gently squeeze the long sides of the plate frame before washing to ensure all strips remain securely remain in the frame.

- Empty plate contents. Using a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty the plate contents. Repeat the procedure two more times for a total of **THREE** washes. Pat dry onto paper towels or other absorbent material.

Note: For automated washing, aspirate all wells and wash **THREE** times with Wash Buffer, overfilling wells with Wash Buffer. Pat dry onto paper towels or other absorbent material.

Streptavidin-HRP Solution Preparation and Incubation:

- *Prepare Streptavidin-HRP Solution* ***immediately before use.***

- Do not store prepared Streptavidin-HRP Solution.

- Use a 15 ml plastic tube to prepare Streptavidin-HRP Solution

- If using a multichannel pipettor **use a new reagent reservoir and new pipette tips** when adding the prepared Streptavidin-HRP Solution.

1) Briefly centrifuge Streptavidin-HRP Concentrate to force entire vial contents to the bottom.

2) **(PP)** Use only the Streptavidin-HRP Solution amount required for the number of strips being used. For each strip, mix 2.5 µl of Streptavidin-HRP Concentrate with 1 ml of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8° C.

For one complete 96-well plate, add 30 µl of Streptavidin-HRP Concentrate to 12 ml of Streptavidin-HRP Dilution Buffer and mix gently.

3) Add 100 µl of prepared Streptavidin-HRP Solution to each well.

4) Carefully attach a new adhesive plate cover, making sure all edges and strips are sealed tightly. Incubate the plate for 30 minutes at room temperature, 20-25°C.

5) Carefully remove the adhesive plate cover, discard plate contents and wash THREE times as described in the Plate Washing section.

Substrate Incubation and Stop Step:

- Use **different disposable reagent reservoirs** when adding the TMB Substrate Solution and Stop Solution.
- Dispense from the bottle ONLY the amount required for the number of strips you are running, 100 µl per well, for the number of wells being used. **Do not use a glass pipet** to measure the TMB Substrate Solution.
- **(PP) Do not combine leftover substrate** with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate Solution.

Pipette 100 µl of TMB Substrate Solution into each well. Allow color reaction to develop at room temperature in the dark for 30 minutes. **Do not cover the plate with aluminum foil or a plate sealer**. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.

After 30 minutes, stop the reaction with the Stop Solution provided, adding 100 µl to each well.

Absorbance Measurement:

Evaluate the plate within 30 minutes of stopping the reaction. Measure the absorbance on an ELISA plate reader set at 450 and 550 nm. Substract 550 nm values from 450 nm values to correct for optical imperfections in the microplate. If 550 nm is not available, measure at 450 nm only. Omitting the 550nm measurement will result in higher absorbance values.

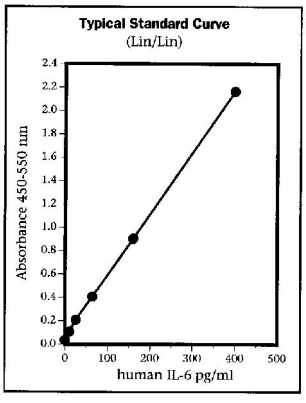
Calculation of Results

a) The standard curve is used to determine the amount of IL-6 in an unknown sample. The standard curve is generated by plotting the average absorbance obtained for each of the Standard concentrations on the vertical (Y) axis versus the corresponding IL-6 concentration on the horizontal (X) axis.

b) Calculate results using graph paper or with a curve-fitting statistical software. The amount of IL-6 in each sample is determined by interpolating from the absorbance value (Y axis) to the IL-6 concentration (X axis) using the standard curve.

c) If a dilution was performed on a test sample, multiply the value interpolated from the standard curve by the dilution factor to calculate the pg/ml of IL-6 in the sample.

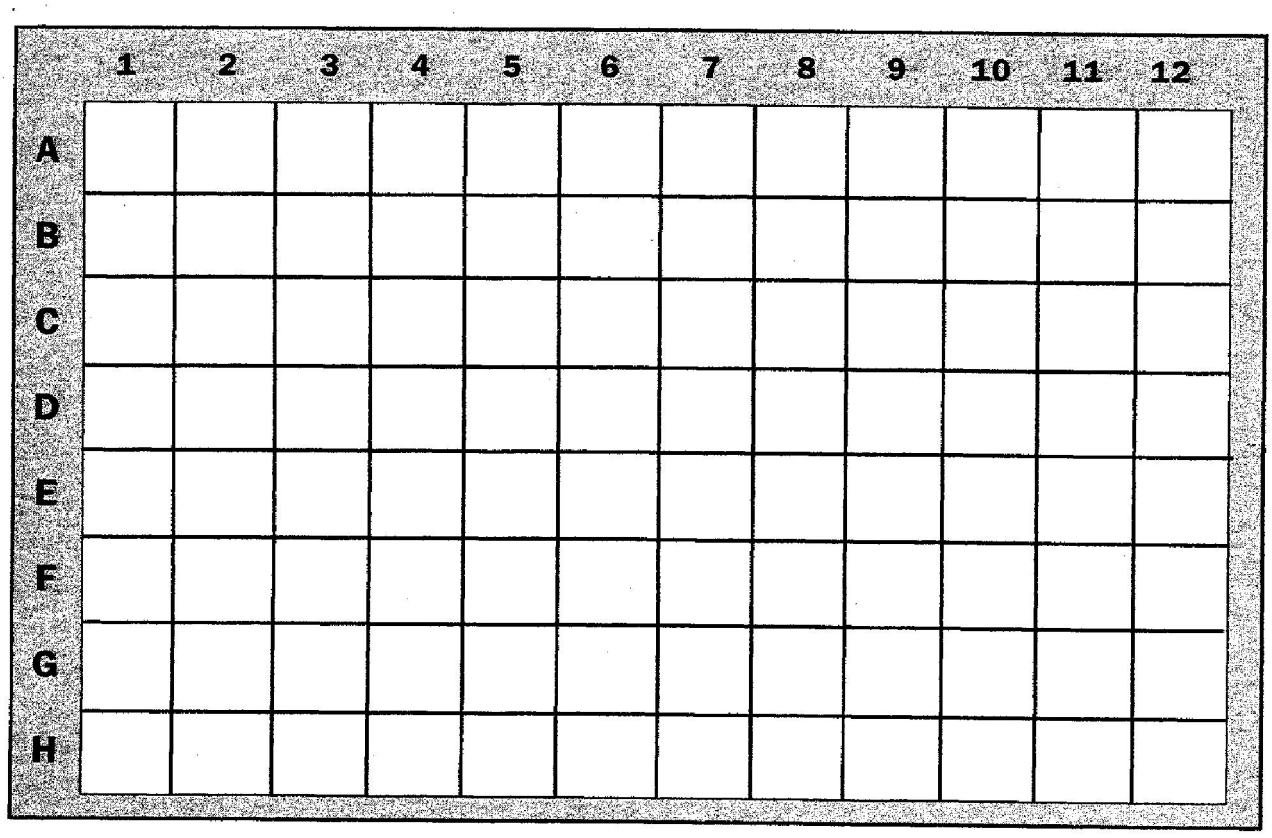
d) Absorbance values obtained for duplicates should be within 10% of the mean value. **Duplicate values that differ from the mean by greater than 10% should be considered suspect and should be repeated.**

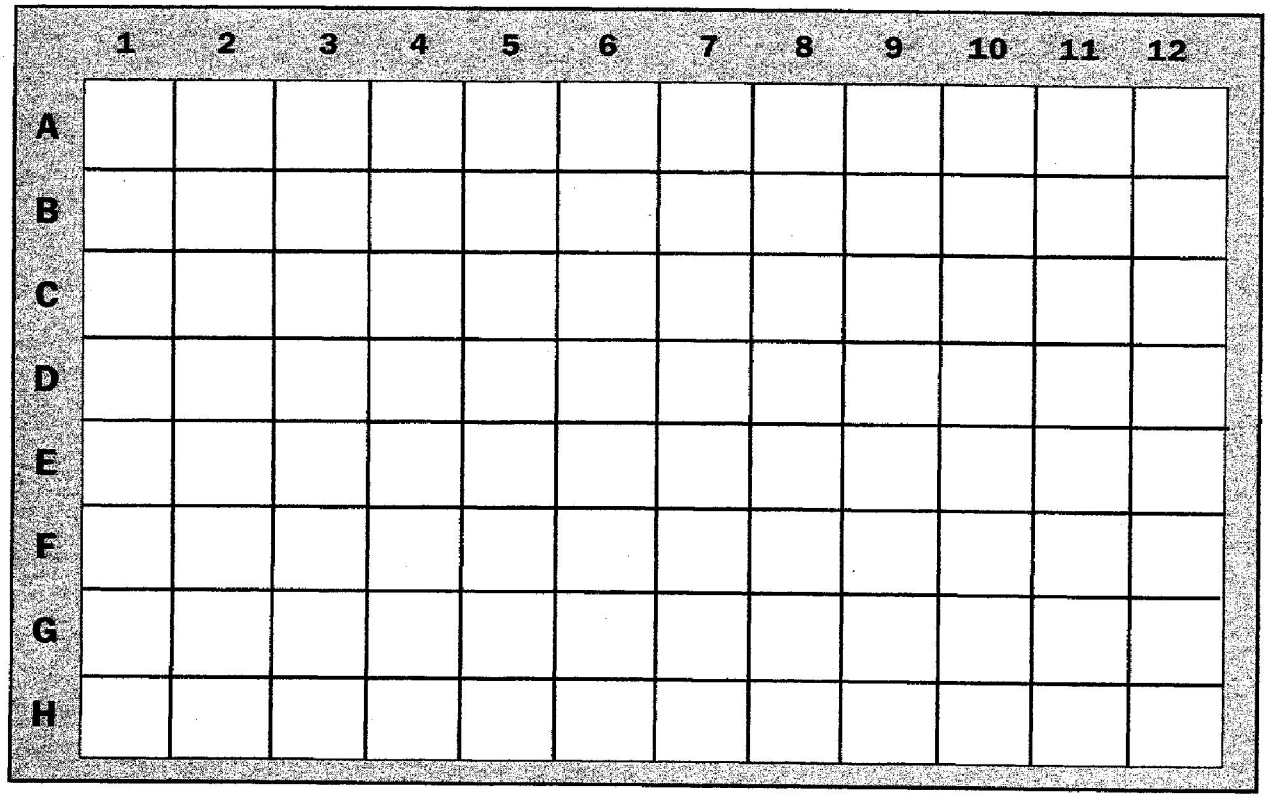


Reference

1. Immunoassay: A Practical Guide, Chan and Perlstein, Eds., 1987, Academic Press: New York, p71.

Two Data Templates

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