Human sIL-2R ELISA Kit

EH2IL2R EH2IL2R2 EH2IL2R5

Number	Description
EH2IL2R	**Human Soluble Interleukin-2 Receptor (sIL-2R) ELISA,** sufficient reagents for 96 determinations
EH2IL2R2	**Human Soluble Interleukin-2 Receptor (sIL-2R) ELISA,** sufficient reagents for 2 × 96 determinations
EH2IL2R5	**Human Soluble Interleukin-2 Receptor (sIL-2R) ELISA,** sufficient reagents for 5 × 96 determinations

Kit Contents	EH2IL2R	EH2IL2R2	EH2IL2R5
Anti-human sIL-2R Precoated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Human sIL-2R Standards	2 vials	4 vials	10 vials
Sample Diluent	6mL	2 × 6mL	5 × 6mL
Conjugate Reagent	6mL	2 × 6mL	5 × 6mL
20X Wash Buffer	55mL	2 × 55mL	5 × 55mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
Adhesive plate covers	2 each	4 each	10 each

For research use only. Not for use in diagnostic procedures.

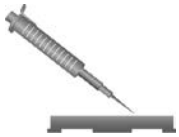
Storage: Upon receipt store the kit at 2-8°C.

Table of Contents

Introduction

The Invitrogen™ Human Soluble Interleukin-2 Receptor (sIL-2R) ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human sIL-2R in serum; EDTA, sodium citrate and heparin plasma and culture supernatants.

Procedure Summary

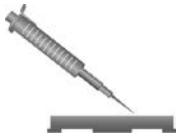


1. Add 50µL of Standards or samples to each well in duplicate.

2. Add 50µL of Conjugate Reagent to each well.

3. Cover plate and incubate at room temperature (20-25°C) for 2 hours on a rotator set at 150 (±10) RPM.

4. Wash plate THREE times.



5. Add 100µL of TMB Substrate to each well.

6. Incubate plate in the dark at room temperature for 30 minutes.

7. Stop reaction by adding 100µL of Stop Solution to each well.

8. Measure absorbance on a plate reader at 450nm or 450 minus 550nm and calculate results.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-l000µL
- Plastic pipettes to deliver 5-15mL
- Ultrapure water
- A glass or plastic one-liter container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs, 3 each
- A rotator set at 150 (±10) RPM
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance can be measured at 450nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

Precautions

- **All samples and reagents must be at room temperature (20-25°C) before use in the assay.**
- Review all instructions carefully and verify all components against the Kit Contents list (page 1) before beginning.
- Sodium azide inactivates the Conjugate Reagent. DO NOT USE SAMPLES CONTAINING SODIUM AZIDE IN THIS ASSAY.
- Do not use a water bath to thaw samples. Thaw samples at room temperature.
- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- 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.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.

- Do not mix reagents from different kit lots. Discard unused ELISA components after assay completion.
- Do not use glass pipettes to measure TMB Substrate. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Crystals may form in the 20X Wash Buffer during storage. Dissolve crystals before diluting by warming in a 36-38°C water bath and mixing.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.

Additional Precaution for the 2-plate and 5-plate Kits

- Dispense, pool, and equilibrate to room temperature only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.

Sample Preparation

Sample Handling

- Serum; EDTA, sodium citrate and heparin plasma; or culture supernatants may be tested in this ELISA.
- 50µL per well of serum, plasma or culture supernatant are required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C.
- Avoid repeated freeze-thaw cycles when storing samples. Mix samples by gently inverting tubes.
- Test samples and standards must be assayed in duplicate each time the assay is performed.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use a heated water bath to thaw or warm samples.
- 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.

Sample Dilution

- If the human sIL-2R concentration possibly exceeds the highest point of the standard curve (i.e., ~6500U/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 or plasma prepare the serial dilutions using the Sample Diluent provided. For example, a 10-fold dilution is prepared by adding 50µL of test sample to 450µL of appropriate diluent. Mix thoroughly between dilutions before assaying.

Reagent Preparation

Look for **(PP)** throughout these instructions for procedural differences when using partial plates.

Wash Buffer

1. Label a clean glass or plastic 1L container “Wash Buffer.”
2. Add the entire contents of the 20X Wash Buffer bottle to the 1L container and dilute to a final volume of 1L with ultrapure water. Mix thoroughly.

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

   Note: Crystals may form in the 20X Wash Buffer during storage. Dissolve crystals before dilution by warming in a 36-38°C water bath and mixing.

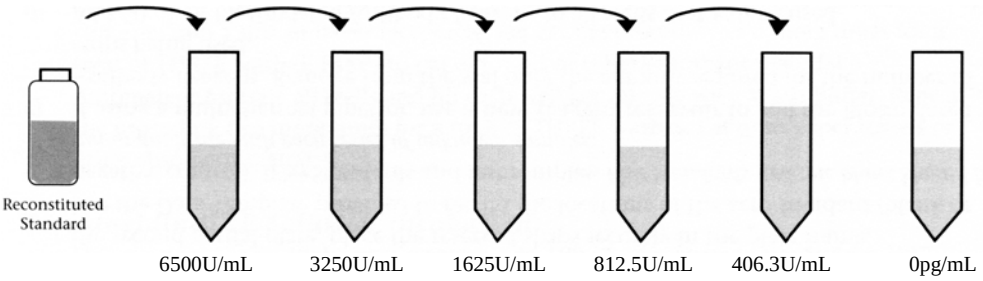
   Note: Wash buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

Standards

1. Reconstitute one vial of Lyophilized Standard per partial plate. See the vial label for the reconstitution volume.
2. Let stand for 10 minutes to dissolve completely. Gently swirl vial to mix.

   Note: Reconstituted standards may be stored at 2-8°C for up to 7 days. If reconstituted standards are not used within 7 days, store at -70°C until kit expiration date.
3. Label six tubes, one for each standard curve point: 6500U/mL, 3250U/mL, 1625 U/mL, 812.5U/mL, 406.3U/mL and 0U/mL, then prepare 1:2 serial dilutions for the standard curve as follows:
4. Pipette 200µL of appropriate diluent into each tube.
5. Pipette 200µL of the reconstituted standard into the first tube (i.e., 6500U/mL) and mix.
6. Pipette 200µL of this dilution into the second tube (i.e., 3250U/mL) and mix.
7. Repeat the serial dilutions (using 200µL) three more times to complete the standards.

Serial dilutions using 200µL



Assay Procedure

A. Standard, Sample and Conjugate Reagent Incubation

- **(PP)** Determine number of strips required. Leave these strips in the plate frame. Tightly seal unused strips in the provided foil pouch with desiccant and store at 2-8°C. After completing 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), human sIL-2R standards and test samples. Perform five standard points and one blank in duplicate with each series of unknown samples.
- If using a multichannel pipettor, use a new reagent reservoir to add the Conjugate 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 Conjugate Reagent.
- **(PP)** Do not combine leftover Conjugate Reagent with that reserved for the other partial plate.

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

   Note: If the human sIL-2R concentration in any test sample possibly exceeds the highest point on the standard curve, 6500U/mL, see Sample Preparation – Sample Dilution Section.
2. Add 50µL of Conjugate Reagent to each well.
3. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over edges and down each strip. Incubate for two (2) hours at room temperature, 20-25°C, on a rotator set at 150 (±10) RPM.
4. Carefully remove adhesive plate cover. Wash plate THREE times with Wash Buffer as described in the Plate Washing section (section B).

B. Plate Washing

1. Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.
2. Discard plate contents. Use a squirt wash bottle to vigorously fill each well completely with Wash Buffer, then discard plate contents. Repeat procedure two additional times for a total of THREE washes. Blot plate 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. Blot plate onto paper towels or other absorbent material.

C. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate Solution and Stop Solution.
- Dispense from bottle ONLY amount required, 100µL per well, for the number of wells being used. Do not use a glass pipette 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.

1. Pipette 100µL of TMB Substrate Solution into each well.
2. Allow enzymatic color reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
3. After 30 minutes, stop the reaction by adding 100µL of Stop Solution to each well.

D. Absorbance Measurement

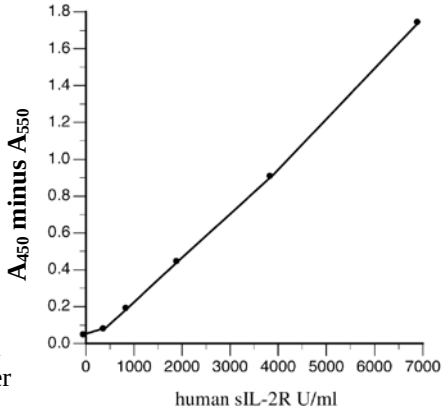
- **Evaluate the plate within 30 minutes of stopping the reaction.** Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If an absorbance at 550nm is unattainable, measure at 450nm only.

  Note: When the 550nm measurement is omitted, absorbance values will be higher.

E. Calculation of Results

- Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding human sIL-2R concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. The human sIL-2R amount in each sample is determined by interpolating from the absorbance value (Y-axis) to human sIL-2R concentration (X-axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate U/ml of human sIL-2R in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.

Example Standard Curve



Performance Characteristics

Sensitivity: < 24U/mL
The sensitivity or lower limit of detection (LLD)[1] was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Specificity:
This ELISA is specific for the measurement of natural and recombinant human sIL-2R. It does not cross react with human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, GM-CSF, IFNγ, IFNα, TNFβ, or mouse IL-2.

Calibration: The standards in this ELISA are calibrated to an internal sIL-2R reference preparation of supernatant from a transfected cell line that secretes recombinant soluble IL-2R. We define 1000 units as the amount of sIL-2R present in 1mL of an internal reference preparation. Units/mL can be converted to sIL-2R pg/mL using the following formula:

$$pg/mL = \frac{value(units/mL)}{0.113}$$

Expected Values: Serum and plasma samples were collected from healthy individuals and evaluated in this assay. The levels of sIL-2R found in these samples are indicated in Table 1.

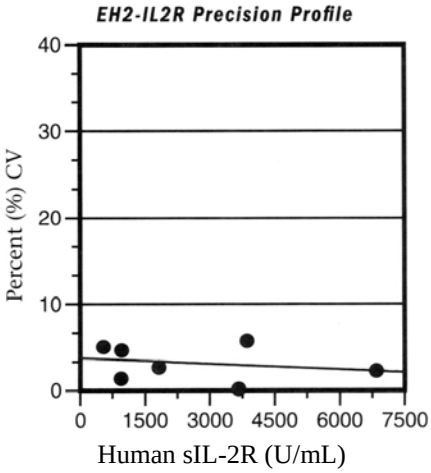
Table 1. Human sIL-2R levels from apparently healthy individuals.

Sample	Average	Range
Serum (n=18)	521U/mL	269-1116U/mL
Plasma (n=20)	642U/mL	342-1521U/mL

Assay Range: 0 to 6500U/mL
Unit values of sIL-2R in Prediluted Standards will vary slightly depending on the kit lot.

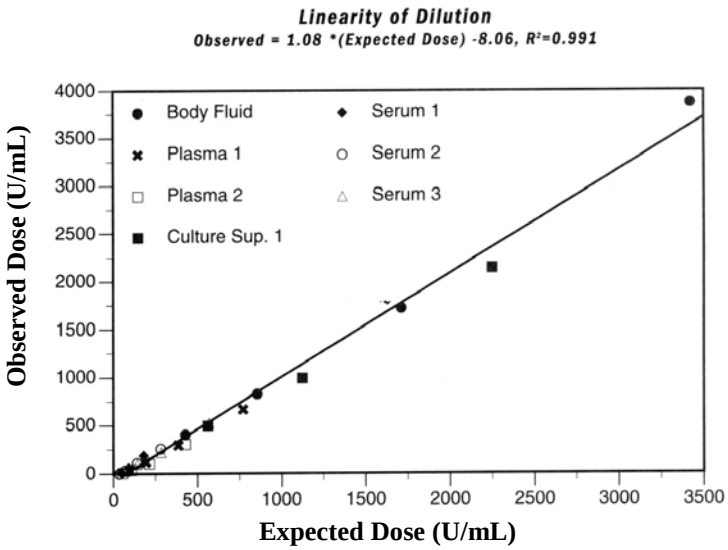
Reproducibility:
Intra-assay CV: < 10%
Inter-assay CV: < 10%

Precision: Typical intra-assay coefficient of variation is plotted against sIL-2R concentration (U/ml). The points represent samples evaluated in duplicate.



Recovery: Recovery was determined by spiking 1.487U/mL natural human sIL-2R into 19 plasma samples and 20 serum samples collected from apparently healthy individuals, and a Sample Diluent control buffer. Endogenous sIL-2R levels were subtracted from the spiked values. Mean serum recovery was 121% and mean plasma recovery was 126%.

Linearity of Dilution: Dilution linearity was determined by serially diluting nine different positive samples. The dilutions were evaluated in the ELISA and the "observed" doses are plotted against the "expected" doses.



Cited Reference

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

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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Data Templates

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B												
C												
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G												
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