DRG® Interleukin-2 Receptor ELISA (EIA-4835)

Revised 20 May 2010 rm (Vers. 3.0) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

The human sIL-2 Receptor ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human sIL-2R.
The human sIL-2R ELISA is for in vitro use. Not for use in therapeutic procedures.

SUMMARY

Interleukin-2 (IL-2) has been described as a factor that promotes the growth and proliferation of human T-cells (17) thus occupying a pivotal role in the generation of the immune response. This proliferation of T lymphocytes is triggered by the interaction of IL-2 with its specific cell surface receptor following T lymphocyte activation. The receptor for IL-2 is composed of at least three distinct polypeptide subunits, the the IL-2R α, IL-2R β, and the IL-2R γ chains giving rise to a 55-65 kD membrane bound protein
The genes encoding IL-2 and these three receptor subunits have been cloned and their complete primary structures have been deduced. Evidence has accumulated that suggests a critical role of IL-2R β in IL-2 signal transduction. IL-2R coupled tyrosine kinases are believed to play a crucial role in that signal transduction.
In addition to the de-novo expression of IL-2R by activated peripheral blood T-cells, a released and fully soluble form of IL-2R (sIL-2 Receptor) has been detected. It was shown that sIL-2 Receptor is present *in vivo*, at low levels in the sera of healthy persons and at markedly elevated levels in various pathological conditions like neoplastic disorders. The structural, functional and molecular characteristics of sIL-2 Receptor have been extensively investigated. By virtue of its ability to bind IL-2, this soluble molecule plays a role in the regulation of the immune response. The detection and quantitation of sIL-2 Receptor provides clinicians with a useful and simple means of assessing immune function *in vivo* as part of the investigation, management and prognosis of a broad spectrum of human diseases.

- hematologic malignancies (neoplasias):
Soluble IL-2R levels are significantly elevated in the sera of patients with adult T-cell leukemia, hairy cell leukemia, lymphocytic leukemia, Hodgkin's and Non-Hodgkin's lymphoma, liver, breast and lung cancer.

- autoimmune or inflammatory diseases:
In patients with systemic lupus erythematosus, rheumatoid arthritis, progressive systemic sclerosis, polymyositis, Kawasaki disease, multiple sclerosis and Diabetes type I plasma concentrations of sIL-2 receptor are significantly elevated as compared to controls.

- infectious diseases:
Both viral and mycobacterial infections lead to high levels of soluble
IL-2R found in Hepatitis, HIV-1, infectious mononucleosis, measles, leprosy, tuberculosis as well as in malaria.

- transplantation or rejection:
sIL-2R turned out to be a very useful marker for monitoring of transplant recipients who show significant elevations of sIL-2R serum levels experiencing rejection episodes in kidney, lung, heart and liver transplantations.

- chronic renal failure or dialysis:
Impaired renal function elevates serum sIL-2R as this molecule is actively transported into the urine.

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- skin:
sIL-2R is significantly elevated in patients with thermal injury.

- therapy:
Recombinant IL-2 as a cancer therapeutic can provoke release of sIL-2R.

For literature update refer to drg@drg-diagnostics.de

PRINCIPLE OF THE TEST

An anti-human sIL-2 Receptor coating antibody is adsorbed onto microwells.
Human sIL-2 Receptor present in the sample or standard binds to antibodies adsorbed to the microwells; a HRP-conjugated anti-human sIL-2 Receptor antibody is added and binds to human sIL-2 Receptor captured by the first antibody.
Following incubation unbound HRP-conjugated anti-human sIL-2 Receptor is removed during a wash step and substrate solution reactive with HRP is added to the wells.
A coloured product is formed in proportion to the amount of human sIL-2R present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human sIL-2 Receptor standard dilutions and human sIL-2 Receptor sample concentration determined.

REAGENTS PROVIDED

1 aluminium pouch with **a Microwell Plate coated** with monoclonal antibody to human sIL-2 Receptor.

1 vial (100 µL) **HRP-Conjugate** anti-human sIL-2 Receptor monoclonal antibody.

2 vials human **sIL-2 Receptor Standard** lyophilized 40 ng/ml upon reconstitution.

1 vial (12 ml) **Sample Diluent**.

1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA).

1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20).

1 vial (15ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml) **Blue-Dye**

1 vial (0.4 ml) **Green-Dye**

2 **adhesive films**

STORAGE INSTRUCTIONS

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

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SPECIMEN COLLECTION

Cell culture supernatant, serum, plasma (EDTA, citrate, heparin), were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or red cells, respectively, as soon as possible after clotting and separation.

Pay attention to a possible **"Hook Effect"** due to high sample concentrations (see chapter 11).
Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
Clinical samples should be kept at 2°C to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive sIL-2 Receptor. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 5 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis.

PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for in vitro use and are not for use in therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
- Exposure to acids will inactivate the conjugate.
- Glass-distilled or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

PREPARATION OF REAGENTS

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

Wash Buffer (1x)

Pour entire contents (50 ml) of the **Wash Buffer Concentrate (20x)** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.
Transfer to a clean wash bottle and store at 2°C to 25°C. Please note that the Wash Buffer (1x) is stable for 30 days.

Wash Buffer (1x) may be prepared as needed according to the following table:

Number of Strips	Vash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

Assay Buffer (1x)

Pour the entire contents (5 ml) of the **Assay Buffer Concentrate** (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.

Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

HRP-Conjugate

Please note that the HRP-Conjugate should be used within 30 minutes after dilution.
Make a 1:100 dilution of the concentrated **HRP-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

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Number of Strips	HRP-Conjugate (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Human sIL-2 Receptor Standard

Reconstitute **human sIL-2R standard** by addition of distilled water.
Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilisation (concentration of reconstituted standard = 40 ng/ml).
Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
After usage remaining standard cannot be stored and has to be discarded.
Standard dilutions can be prepared directly on the microwell plate (see 10.c) or alternatively in tubes (see 0).

External Standard Dilution

Label 7 tubes, one for each standard point.

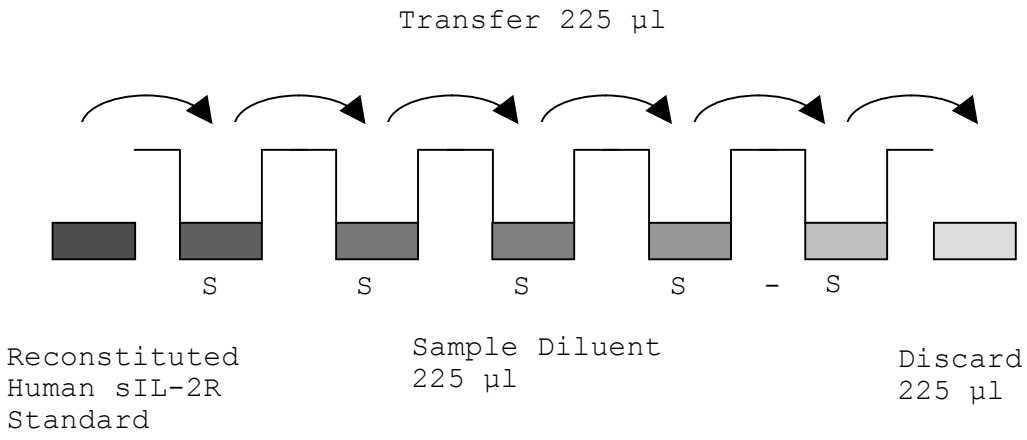
S1, S2, S3, S4, S5, S6, S7

Then prepare 1:2 serial dilutions for the standard curve as follows:
Pipette 225 µl of Sample Diluent into each tube.

Pipette 225 µl of reconstituted standard (concentration = 40 ng/ml) into the first tube, labelled S1, and mix (concentration of standard 1 = 20 ng/ml).
Pipette 225 µl of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.
Repeat serial dilutions 5 more times thus creating the points of the standard curve (see figure 1).

Sample Diluent serves as blank.

Figure 1



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Addition of colour-giving reagents: Blue-Dye, Green-Dye

In order to help our customers to avoid any mistakes in pipetting the ELISAs, a new tool is offered that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent: Before standard and sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

5 ml Diluent	20 µL ***Blue-Dye***
12 ml Diluent	48 µL ***Blue-Dye***
50 ml Diluent	200 µL ***Blue-Dye***

2. HRP-Conjugate: Before dilution of the concentrated HRP-Conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet: Preparation of HRP-conjugate.

3 ml Assay Buffer (1x)	30 µL ***Green-Dye***
6 ml Assay Buffer (1x)	60 µL ***Green-Dye***

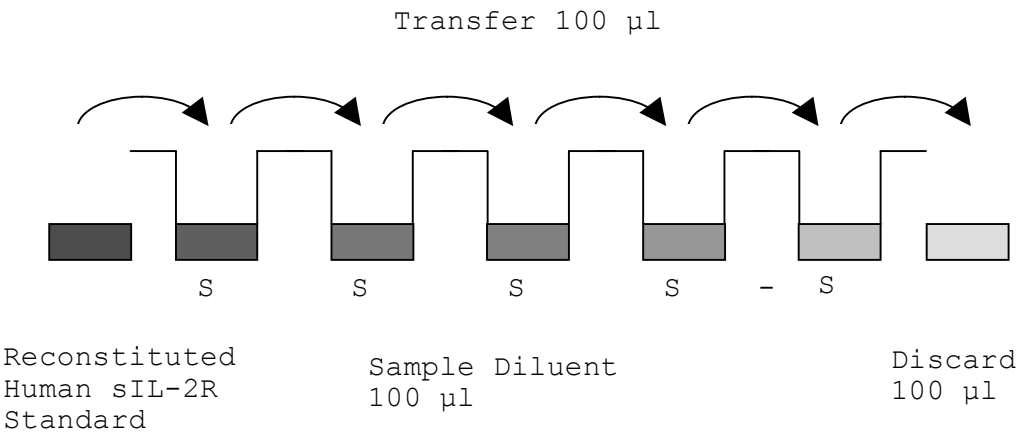
TEST PROTOCOL

a. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

b. Wash the microwell strips twice with approximately 400 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.
After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry**.

c. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 0):
Add 100 µl of Sample Diluent in duplicate to all **standard wells**. Pipette 100 µl of prepared **standard** (see Preparation of Standard 9.4, concentration = 40 ng/ml) in duplicate into well A1 and A2 (see Table 1Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 20 ng/ml), and transfer 100 µl to wells B1 and B2, respectively (see Figure 2). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human sIL-2R standard dilutions ranging from 20.00 to 0.31 ng/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 2:



In case of an **external standard dilution** (see 0), pipette 100 µl of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

Table 1: Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (20 ng/ml)	Standard 1(20 ng/ml)	Sample 1	Sample 1
B	Standard 2 (10 ng/ml)	Standard 2 (10 ng/ml)	Sample 2	Sample 2
C	Standard 3 (5 ng/ml)	Standard 3 (5 ng/ml)	Sample 3	Sample 3
D	Standard 4 (2.5 ng/ml)	Standard 4 (2.5 ng/ml)	Sample 4	Sample 4
E	Standard 5 (1.25 ng/ml)	Standard 5 (1.25 ng/ml)	Sample 5	Sample 5
F	Standard 6 (0.63 ng/ml)	Standard 6 (0.63 ng/ml)	Sample 6	Sample 6
G	Standard 7 (0.31 ng/ml)	Standard 7 (0.31 ng/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 100 µL of **Sample Diluent** in duplicate to the blank wells.

e. Add 50 µL of **Sample Diluent** to all wells designated for samples.

f. Add 50 µL of each **sample**, in duplicate, to the sample wells.

g. Prepare **HRP-Conjugate**. (see Preparation of HRP-Conjugate 9.3.)

h. Add 50 µL of **HRP-Conjugate** to all wells.

i. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 3 hours, if available on a microplate shaker set at 100 rpm.

j. Remove adhesive film and empty wells. **Wash** microwell strips 3 times according to point b. of the test protocol. Proceed immediately to the next step.

k. Pipette 100 µL of **TMB Substrate Solution** to all wells.

l. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point n. of this protocol) before positive wells are no longer properly recordable.
It is recommended to add the stop solution when the highest standard has developed a dark blue colour.
Alternatively the colour development can be monitored by the ELISA reader at 620nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

m. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

n. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the sIL-2 Receptor standards.
Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human sIL-2R concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human sIL-2R for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human sIL-2R concentration.
- **If instructions in this protocol have been followed samples have been diluted 1:2 (50 µl sample + 50 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).**
- **Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human sIL-2R levels (Hook Effect). Such samples require further external predilution according to expected human sIL-2R values with Sample Diluent in order to precisely quantitate the actual human sIL-2R level.**
- It is suggested that each testing facility establishes a control sample of known human sIL-2R concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in figure 3. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for human sIL-2R ELISA. Human sIL-2R was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

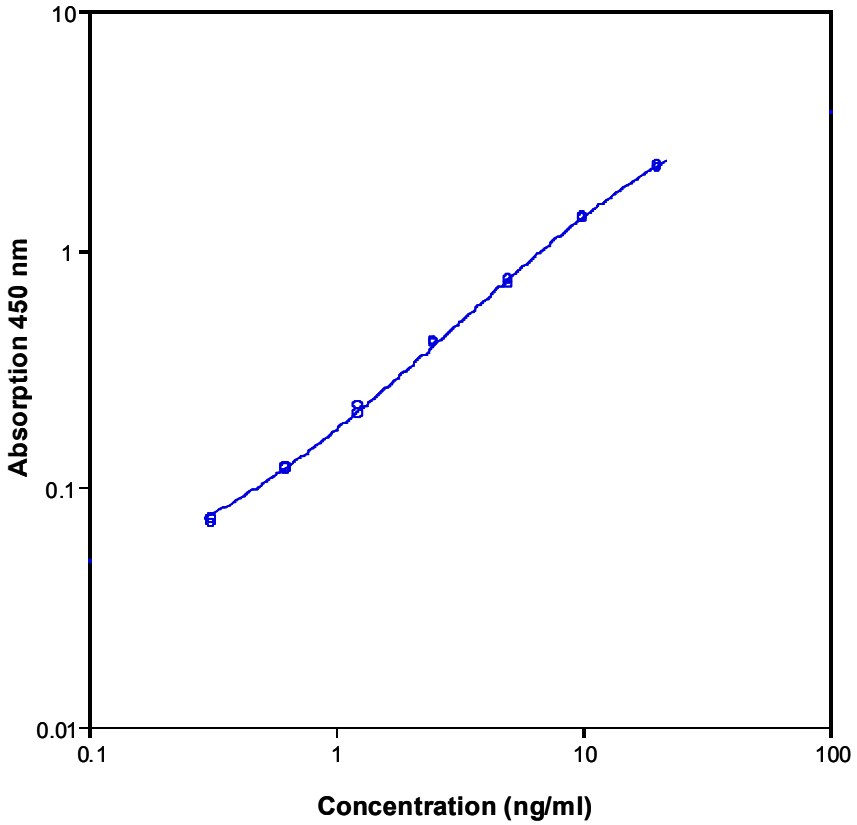


Table 2: Typical data using the human sIL-2 Receptor ELISA
Measuring wavelength: 450 nm, Reference wavelength: 620 nm

Standard	sIL-2 Receptor Conc. (ng/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	20.0	2.239 2.193	2.216	3.25
2	10.0	1.366 1.354	1.360	0.85
3	5.0	0.710 0.747	0.729	2.62
4	2.5	0.403 0.410	0.407	0.49
5	1.25	0.204 0.219	0.212	1.06
6	0.63	0.120 0.122	0.121	0.14
7	0.31	0.071 0.074	0.073	0.21
Blank	0	0.010 0.012	0.011	0.14

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. temperature effects). Furthermore shelf life of the kit may effect enzymatic activity and thus colour intensity. Values measured are still valid.

LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of immunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the Sample Diluent.

PERFORMANCE CHARACTERISTICS

Sensitivity

The limit of detection of human sIL-2R defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.27 ng/ml (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human sIL-2R. 2 standard curves were run on each plate. Data below show the mean human sIL-2R concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 7.2%.

Table 3: The mean human sIL-2R concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human sIL-2R Concentration (ng/ml)	Coefficient of Variation (%)
1	1	2.60	11
	2	2.53	7
	3	2.01	3
2	1	1.47	10
	2	1.54	7
	3	1.32	5
3	1	1.47	9
	2	1.56	7
	3	1.30	9
4	1	1.72	2
	2	1.80	7
	3	1.64	7
5	1	1.43	8
	2	1.52	11
	3	1.33	4
6	1	3.05	10
	2	3.42	5
	3	2.67	4

Inter-Assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human sIL-2R. 2 standard curves were run on each plate. Data below show the mean human sIL-2R concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 9.8%.

Table 4: The mean human sIL-2R concentration and the coefficient of variation of each sample

Sample	Mean Human sIL-2R Concentration (ng/ml)	Coefficient of Variation (%)
1	2.381	13.6
2	1.440	8.1
3	1.443	9.1
4	1.803	9.2
5	1.430	6.5
6	3.048	12.3

Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human sIL-2R into different pooled normal human serum samples. Recoveries were determined in 3 independent experiments with 4 replicates each.
The amount of endogenous human sIL-2R in unspiked serum was subtracted from the spike values.
The overall mean recovery was 75%.

Dilution Parallelism

4 serum samples with different levels of human sIL-2R were analysed at serial 2 fold dilutions with 4 replicates each.
The recovery ranged from 77% to 107% with an overall recovery of 86% (see Table 5).

Table 5:

Sample	Dilution	Expected Human sIL-2R Concentration (ng/ml)	Observed Human sIL-2R Concentration (ng/ml)	Recovery of Expected Human sIL-2R Concentration (%)
1	1:2	--	1.27	--
	1:4	6.37	5.92	93
	1:8	3.18	2.58	81
	1:16	1.59	1.28	81
2	1:2	--	17.0	--
	1:4	8.50	9.08	107
	1:8	4.25	3.83	90
	1:16	2.13	1.86	87
3	1:2	--	18.6	--

	1:4	9.30	8.35	90
	1:8	4.65	3.69	79
	1:16	2.33	1.78	77
4	1:2	--	16.72	--
	1:4	8.36	6.79	81
	1:8	4.18	3.49	83
	1:16	2.09	1.84	88

Sample Stability

Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human sIL-2R levels determined. There was no significant loss of human sIL-2R immunoreactivity detected by freezing and thawing.

Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human sIL-2R level determined after 24, 48 and 96 h. There was no significant loss of human sIL-2R immunoreactivity detected during storage under above conditions.

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a sIL-2 Receptor positive serum. There was no cross reactivity detected.

Expected Values

A panel of 40 serum as well as EDTA, citrate and heparin plasma samples from randomly selected apparently healthy donors (males and females) was tested for human IL-2R.
The levels measured may vary with the sample collection used.
For detected human IL-2R levels see Table 6.

Table 6:

Sample Matrix	Number of Samples Evaluated	Range (ng/ml)	Mean (ng/ml)	Standard Deviation (ng/ml)
Serum	40	1.9 – 13.1	4.7	2.6
Plasma (EDTA)	40	0.9 – 8.1	2.4	1.3
Plasma (Citrate)	40	1.8 - 7.8	3.8	1.65
Plasma (Heparin)	40	2.12 – 8.0	4.0	1.7

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REAGENT PREPARATION SUMMARY

Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20x (50 ml) to 950 ml distilled water.

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

Assay Buffer (1x)

Add **Assay Buffer Concentrate** 20x (5 ml) to 95 ml distilled water.

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

HRP-Conjugate

Make a 1:100 dilution of **HRP-Conjugate** in Assay Buffer (1x):

Number of Strips	HRP-Conjugate (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Human sIL-2R Standard

Reconstitute lyophilized **human sIL-2R standard** with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

TEST PROTOCOL SUMMARY

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µl Sample Diluent, in duplicate, to all standard wells. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 100 µl from well to well. Discard 100 µl from the last wells.
   Alternatively external standard dilution in tubes (see 0): Pipette 100 µl of these standard dilutions in the microwell strips.
4. Add 100 µl Sample Diluent, in duplicate, to the blank wells.
5. Add 50 µl Sample Diluent to sample wells.
6. Add 50 µl sample in duplicate, to designated sample wells.

7. Prepare HRP-Conjugate.
8. Add 50 µl HRP-Conjugate to all wells.
9. Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C).
10. Empty and wash microwell strips 3 times with Wash Buffer.
11. Add 100 µl of TMB Substrate Solution to all wells.
12. Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
13. Add 100 µl Stop Solution to all wells.
14. Blank microwell reader and measure colour intensity at 450 nm.

Note: If instructions in this protocol have been followed samples have been diluted 1:2 (50 µl sample + 50 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità