GE Healthcare

Amersham Interleukin-1β [(r)IL-1β], Rat Biotrak ELISA System

96 determinations

Product Booklet

Code: RPN2743

Page finder

1. Legal

http://www.gelifesciences.com

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2. Handling

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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 statement(s) for specific advice.

2.2. Stability and storage

a. Store all reagents at 2–8°C upon receipt. Do not freeze reagents.

b. Refer to the expiry date stamped on the kit box.

c. Do NOT use kit beyond the stated expiry date.

3. Materials

3.1. Materials provided

- Anti-rat IL-1β precoated stripwell plate, 1 (96 well)
- Recombinant rat IL-1β standard, lyophilized, 2 vials
- Sample diluent, 1 vial (12 ml), containing 0.1% sodium azide.
- Biotinylated antibody reagent, 1 vial (12 ml), containing 0.1% sodium azide.
- 30× Wash buffer, 1 vial (50 ml)
- Streptavidin-HRP concentrate, 1 vial (0.075 ml)
- Streptavidin-HRP dilution buffer, 1 vial (13 ml)
- Premixed TMB substrate solution, 1 vial (12–13 ml)
- Stop solution, 1 vial (13–15 ml), <1% sulfuric acid
- Adhesive plate covers, 6
- Instruction booklet, 1

3.2. Additional materials required

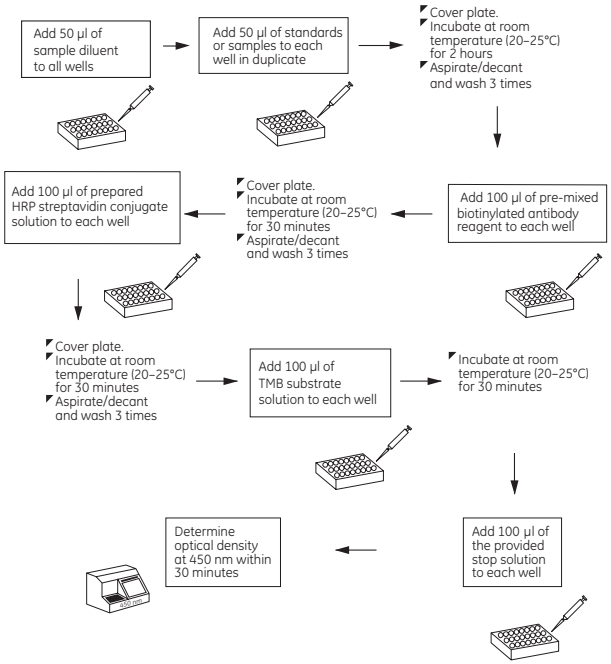
- Precision pipettes with disposable plastic tips to deliver 5–1000 µl.
- Plastic pipettes to deliver 5–15 ml.
- Distilled or deionized water for wash buffer and standard reconstitution.
- A glass or plastic 2 litre container to prepare wash buffer.
- A squirt wash bottle, or an automated immunoplate washer.
- 1.5 ml polypropylene or polyethylene tubes to prepare standards. Do not use polystyrene, polycarbonate or glass tubes.
- 4 disposable reagent reservoirs.
- 15 ml plastic tube to prepare streptavidin-HRP solution.

- A microcentrifuge for preparing streptavidin-HRP solution.
- A standard ELISA reader for measuring absorbance at 450 nm and 550 nm. If a 550 nm filter is not available, the absorbance can be read only at 450 nm. Refer to the instruction manual supplied with the instrument used.
- Graph paper or a computerized curve-fitting statistical software package.

4. Introduction

The Biotrak™ rat interleukin-1beta (IL-1β) ELISA from GE Healthcare is an enzyme-linked immunosorbent assay for the quantitative measurement of rat IL-1β in serum; EDTA, heparin and sodium citrate plasma; and culture supernatant.

5. Summary of procedure



6. Critical parameters

The following points are critical:

- All specimens and reagents must be at room temperature (20–25°C) before use in the ELISA.
- Vigorous washing of plate is essential.
- Sample diluent must be used when diluting all samples.
- Do not use a 37°C water bath to thaw samples. Thaw samples at room temperature.
- If using a multichannel pipette, always use a new disposable reagent reservoir for the addition of each reagent.
- Use fresh disposable pipette tips for each transfer to avoid cross contamination.
- Use a new adhesive plate cover for each incubation step in the ELISA.
- Do not mix reagents from different kit lots.
- 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.
- If using samples that are clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any question about the integrity of a sample, make a note and interpret results with caution.
- Individual components of this assay may contain antibiotics and preservatives. Gloves must be worn while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Do not use glass pipettes to measure the TMB substrate solution.

- Care must be taken not to contaminate the TMB substrate solution. If the solution is blue prior to use, **DO NOT USE.**
- 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.

7. Sample preparation

7.1. Handling and storage

Serum; EDTA, heparin and sodium citrate plasma; and culture supernatant may be tested in this ELISA.

- 50 µl of sample or standard per well is required in this assay.
- Samples that are to be assayed within 24 hours should be stored at 2–8°C. When storing samples for longer periods of time, dispense into aliquots and freeze them at -70°C.
- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Avoid repeat freeze thaw cycles when storing samples.
- Bring samples gradually to room temperature before running the assay. **Do not use heated water baths to thaw or warm samples.**
- Mix samples by gently inverting the tubes.
- If samples are clotted, grossly hemolyzed, lipemic or microbially contaminated, make a note and interpret results with caution.

7.2. Sample dilution

If the rat IL-1β concentration of a sample exceeds the highest point of the standard curve, 2500 pg/ml, prepare one or more five-fold dilutions of the test sample. For example, a five-fold dilution is prepared by adding 50 µl of test sample to 200 µl of sample diluent. Mix thoroughly between dilutions before assaying. Prepare all sample dilutions using the sample diluent provided.

8. Reagent preparation

8.1. Wash buffer

Label a clean glass or plastic 2 litre container 'wash buffer.' 30× wash buffer may have a cloudy appearance. Add the entire contents of the 30× wash buffer bottle to the 2 litre container and dilute to a final volume of 1.5 litres with distilled or deionized water. Mix thoroughly. **Wash buffer must be at room temperature** prior to use in the assay. If running partial plates, store the reconstituted wash buffer at 2–8°C. **Do not use wash buffer if it becomes visibly contaminated during storage.**

8.2. Standards

a. Two vials of lyophilized standard are provided with this kit. Reconstitute and use one vial per partial plate.

b. Prepare standards shortly before use. Use within the same day as reconstitution. **Do not store reconstituted standards.**

c. Reconstitute standard in distilled or deionized water. The reconstitution volume is stated on the standard vial label. Mix by gently inverting the vial until the contents have completely dissolved. Use the sample diluent provided to prepare the standard curve dilutions. See step e) below for further instructions.

d. Label 7 tubes, one for each standard curve point: 2500, 1000, 400, 160, 64, 25.6 and 0 pg/ml. Prepare serial dilutions for the standard curve as follows:

e. Into the 2500 pg tube pipette 250 µl of sample diluent.

f. Into the remaining tubes pipette 240 µl of sample diluent.

g. Pipette 250 µl of the reconstituted standard into the first tube, 2500 pg/ml and mix.

h. Pipette 160 µl of this dilution into the next tube, 1000 pg/ml and mix.

i. Repeat serial dilutions four more times to complete the standard curve.

8.3. Streptavidin-HRP solution

a. Prepare streptavidin-HRP solution **no more than 15 minutes prior to use.** Do not prepare more than required. See step e) below when running partial plates.

b. Do not store prepared streptavidin-HRP solution.

c. Use a 15 ml plastic tube to prepare the streptavidin-HRP solution. Centrifuge the streptavidin-HRP concentrate to force the entire contents to the bottom of the tube.

d. Add 30 µl of streptavidin-HRP concentrate to 12 ml streptavidin-HRP dilution buffer and mix gently. This is the streptavidin-HRP solution.

e. When running partial plates, use only what is required for the number of strips being run. To run a half plate, add 15 µl streptavidin-HRP concentrate to 6 ml streptavidin-HRP dilution buffer, mix gently.

9. Assay procedure

9.1. Sample and biotinylated antibody reagent incubation

a. Determine the number of strips required. Leave these in the plate frame. Place the remaining unused strips back in the foil pouch with the desiccant provided. Store the reserved strips at 2–8°C, making sure the foil pouch is tightly sealed. After running the assay, retain the plate frame for the second partial plate. When running the second partial plate, place the reserved strips securely in the plate frame.

b. Record the locations of the rat IL-1β standards and test samples. Six standards and one zero must be run in duplicate with each series of unknown samples.

c. Add 50 µl sample diluent to each well. Add 50 µl standard or sample to each well in duplicate. **If the IL-1β concentration in any test sample exceeds the highest point on the standard curve,** 2500 pg/ml, see section 7.2. Mix well by gently tapping the plate several times.

d. Carefully cover the plate with an adhesive plate cover. Ensure that all edges and strips are sealed tightly by running a thumb over the edges and down each strip. Incubate for two (2) hours at room temperature, 20–25°C.

e. At the end of the incubation period, carefully remove the adhesive plate cover. Wash the plate THREE times with wash buffer, using the procedure outlined in section 9.2.

f. If using a multichannel pipette, 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.

g. Add 100 µl of the biotinylated antibody reagent to all wells being used.

h. Carefully cover the plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running a thumb over the edges and down each strip. Incubate for 30 minutes at room temperature, 20–25°C.

i. At the end of the incubation period, carefully remove the adhesive plate cover. Wash the plate THREE times with wash buffer, using the procedure outlined in section 9.2.

9.2. Washing the plate

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

Manual wash: Decant the contents of the plate into a sink or other receptacle. Using a squirt bottle, vigorously fill each well completely with wash buffer, then decant the contents into a sink or other receptacle. Repeat the procedure two more times for a total of THREE washes. Blot on to paper towels or other absorbent material.

Automated wash: Aspirate all wells and wash THREE times with wash buffer making sure wells are filled with wash buffer. Blot on to paper towels or other absorbent material.

9.3. Streptavidin-HRP solution incubation

a. Add 100 µl of prepared streptavidin-HRP solution, see section 8.3, to each well. If using a multichannel pipette, use a new reagent reservoir and pipette tips when adding the prepared streptavidin-HRP solution.

b. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate the plate for 30 minutes at room temperature, 20–25°C.

c. At the end of the incubation, carefully remove the adhesive plate cover, decant the plate then wash THREE times using the procedure outlined in section 9.2.

9.4. Substrate incubation and stop step

a. Use new disposable reagent reservoirs and pipette tips when adding the TMB substrate solution and stop solution.

b. Dispense from the bottle ONLY the amount required for the number of strips being run, 100 µl per well. **Do not use a glass pipette** to measure the TMB substrate solution. Do not combine leftover substrate with that reserved for the second half plate. Care must be taken to ensure that the remaining TMB substrate solution is not contaminated.

c. Pipette 100 µl of TMB substrate solution into each well.

d. Allow enzymatic color reaction to develop at room temperature 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.

e. After 30 minutes, stop the reaction by adding 100 µl of stop solution to each well.

9.5. Reading the absorbance

Measure the absorbance on an ELISA reader set at 450 and 550 nm. Subtract readings of 550 nm from the readings at 450 nm. Reading at dual wavelengths will correct for optical imperfections in the microplate. If a wavelength correction is not available read the plate at 450 nm. Note: When the 550 nm adjustment is omitted, OD values will be higher. **THE PLATE MUST BE READ WITHIN 30 MINUTES OF STOPPING THE REACTION.**

10. Calculation of results

a. The standard curve is used to determine the amount of rat IL-1β in an unknown sample. The standard curve is generated by plotting the average absorbance (450–550 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-1β concentration on the horizontal (X) axis.

b. Calculate results manually using graph paper or with a curve-fitting statistical software package. If using curve-fitting software, plot a four parameter logistic curve fit. If four parameter is not available, a point-to-point curve fit may be used. The amount of IL-1β in each sample is determined by interpolating the IL-1β concentration (X axis) from the absorbance value (Y axis). If a dilution was performed on a test sample, multiply the value interpolated from the standard curve by the dilution factor to calculate the amount of IL-1β in the sample.

c. Optical density values obtained for duplicates should be within 10% of the mean. **Duplicate values that differ from the mean by greater than 10% should be considered suspect and should be repeated.**

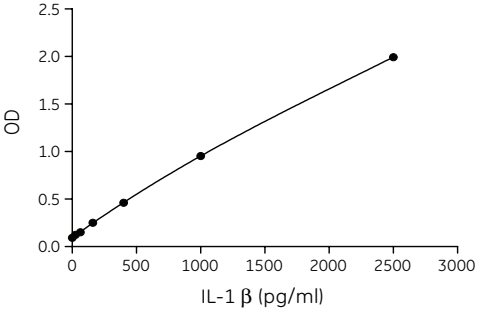


Figure 1. Typical standard curve

11. Performance characteristics

11.1. Sensitivity

12 pg/ml

The sensitivity of this assay, 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.

11.2. Assay range

25.6–2500 pg/ml

Standard curve points are 0, 25.6, 64, 160, 400, 1000 and 2500 pg/ml.

11.3. Reproducibility

Reproducibility of the Biotrak rat IL-1β ELISA was evaluated in each sample matrix. To determine within-assay precision, 20 replicates of samples containing two levels of recombinant rat IL-1β were run on a single plate.

To evaluate between-assay precision, samples were tested by three operators who performed at least three separate assays on more than one day. Twelve duplicate sample values were used to calculate between-assay precision data for each level of IL-1β. Data is shown in table 1.

[1]Immunoassay: A Practical Guide, ed. Chan and Perlstein, p.71, Academic Press, 1987.

Table 1.

		Within-assay precision			Between-assay precision		
Sample	**Level**	**Mean (pg/ml)**	**SD (pg/ml)**	**CV (%)**	**Mean (pg/ml)**	**SD (pg/ml)**	**CV (%)**
Serum	1	901.3	47.7	5.3	927.3	63.3	6.8
	2	187.7	11.4	6.1	219.9	19.4	8.8
EDTA	1	1140.6	64.1	5.6	548.9	38.0	6.9
plasma	2	287.4	20.8	7.2	274.5	24.1	8.8
Citrate	1	801.8	49.8	6.2	867.4	62.3	7.2
plasma	2	301.1	32.7	10.9	364.1	29.1	8.0
Heparin	1	785.7	22.8	2.9	829.7	69.7	8.4
plasma	2	197.5	23.0	11.6	227.3	28.0	12.3
Cell culture	1	817.9	53.9	6.6	884.2	54.7	6.2
supernatant	2	438.9	35.0	8.0	429.4	30.2	7.0

11.4. Specificity

The ELISA is specific for the measurement of natural and recombinant rat IL-1β. It does not cross react with the following cytokines: rat IFNγ, IL-1α, IL-2, IL-4, IL-6, IL-10, MCP-1, MIP-1α and RANTES; human, mouse and pig IL-1γ.

11.5. Expected values

For normal serum and plasma:

Twenty pooled serum; EDTA; heparin and citrate samples collected from apparently healthy Wistar rats were run in this assay. IL-1β levels in 77 of 80 samples tested were below the detection limit of the assay.

11.6. Spike and recovery

Recovery of rat IL-1β was evaluated using the Biotrak rat IL-1β ELISA kit. Pooled serum and plasma samples from Wistar rats and sample diluent controls were spiked with recombinant rat IL-1β. Endogenous IL-1β levels were determined by running unspiked samples along with spiked aliquots of the same samples in the ELISA. Expected values were calculated by adding endogenous IL-1β levels to those of spiked diluent controls. Percent (%) recovery was found by dividing observed by expected values. Results for representative individual samples and populations are shown below and overleaf:

Table 2A.

		Representative sample			
Sample	**Level**	**Expected (pg/ml)**	**Observed (pg/ml)**	**Recovery (%)**	**n**
Serum	1	935.8	939.2	100.4	5
	2	328.9	340.6	103.6	5
EDTA plasma	1	935.8	883.2	94.4	4
	2	328.9	349.2	106.2	4
Citrate plasma	1	1006.7	977.4	97.1	5
	2	343.6	321.8	93.7	5
Heparin plasma	1	1006.7	986.1	98.0	4
	2	343.6	351.3	102.2	4

Table 2B.

		Sample population			
Sample	**Level**	**Expected (pg/ml)**	**Observed (pg/ml)**	**Recovery (%)**	**n**
Serum	1	935.8	898.7	96.0	5
	2	328.9	324.3	98.6	5
EDTA plasma	1	935.8	905.0	96.7	4
	2	328.9	336.7	102.4	4
Citrate plasma	1	1006.7	949.6	94.3	5
	2	343.6	323.1	94.0	5
Heparin plasma	1	1006.7	987.6	98.1	4
	2	343.6	347.1	101.0	4

11.7. Linearity of dilution

Pooled serum and plasma samples from Wistar rats were spiked with recombinant rat IL-1β, serially diluted in sample diluent and evaluated in the Biotrak rat IL-1β ELISA. Linearity of dilution for plasma was assessed for each anticoagulant. Representative data from EDTA plasma samples is shown below. Results for heparin and citrate plasma were similar to those shown for EDTA plasma. Observed values were compared to expected values to calculate % recovery and demonstrate the linearity of dilution of the assay. Data for cell culture medium was generated using natural samples.

Table 3.

Sample	Dilution	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
Serum	Neat	1170.2	1170.2	–
	1:2	585.1	559.5	95.6
	1:4	292.6	275.4	94.1
	1:8	146.3	126.5	86.5
	1:16	73.1	71.1	97.2
EDTA plasma	Neat	1342.7	1342.7	–
	1:2	671.4	610.1	90.9
	1:4	335.7	324.3	96.6
	1:8	167.8	161.0	95.9
	1:16	83.9	72.5	86.
Cell culture medium	Neat	233.3	233.3	–
	1:2	116.7	108.1	92.7
	1:4	58.3	52.8	90.4
	1:8	29.2	25.3	86.9

12. Related products

Biotrak range of Easy ELISA systems

Interferon-alpha [(h)IFNα]	RPN5960
Interferon-gamma [(h)IFNγ]	RPN5961
Interleukin 1β [(h)IL-1β]	RPN5971
Interleukin-2 [(h)IL-2]	RPN5965
Interleukin-2 [(m)IL-2]	RPN5966
Interleukin-6 [(h)IL-6]	RPN5968
Interleukin-8/Neutrophil-activating peptide-1-1 [(h)IL-8/NAP-1]	RPN5969
Interleukin-10 [(m)IL-10]	RPN5963
Interleukin-13 [(h)IL-13]	RPN5972
Monocyte chemoattractant protein-1 [(h)MCP-1]	RPN5964
Transforming growth factor beta-1 [(h)TGFβ_1]	RPN5970
Tumor necrosis factor-alpha [(h)TNFα]	RPN5967

Biotrak range of human cytokine and growth factor ELISA systems

Interleukin-1α [(h)IL-1α]	RPN2750
Interleukin-1β [(h)IL-1β]	RPN2751
Interleukin-6 [(h)IL-6]	RPN2754
Interleukin-8 [(h)IL-8]	RPN2764
Interleukin-10 [(h)IL-10]	RPN2755
Interleukin-12 p40/70 [(h)IL-12]	RPN2765
Interferon-gamma [(h)IFNγ]	RPN2757
Tumor necrosis factor-alpha [(h)TNFα]	RPN2758
Vascular Endothelial Growth Factor [(h)VEGF]	RPN2779

Biotrak range of high sensitivity human cytokine and growth factor ELISA systems

Interferon-gamma [(h)IFNγ]	RPN2787
Interleukin-6 [(h)IL-6]	RPN2784
Interleukin-10 [(h)IL-10]	RPN2785
Tumor necrosis factor-alpha [(h)TNFα]	RPN2788

Biotrak range of mouse cytokine and growth factor ELISA systems

Interferon-gamma [(m)IFNγ]	RPN2707
Interleukin-1β [(m)IL-1β]	RPN2720
Interleukin-4 [(m)IL-4]	RPN2712
Interleukin-6 [(m)IL-6]	RPN2708
Interleukin-12 [(m)IL-12]	RPN2702
Tumor necrosis factor-alpha [(m)TNFα]	RPN2718

Biotrak range of rat cytokine and growth factor ELISA systems

Cytokine-induced neutrophil chemoattractant [(r)GRO/C1NC-1]	RPN2730
Interleukin-6 [(r)IL-6]	RPN2742
Monocyte chemoattractant protein-1 [(r)MCP-1]	RPN2740
Tumor necrosis factor-alpha [(r)TNFα]	RPN2744

Cell Proliferation assay system and reagents

Cell Proliferation Biotrak ELISA	RPN250
Cell proliferation kit for immunocytochemistry	RPN20
Monoclonal anti-bromodeoxyuridine	RPN202
Cell Proliferation Labeling Reagent	RPN201

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