



ELISA Kit

Catalog # **KRC2341 (96 tests)**
 KRC2342 (192 tests)

Rat **IL-18**

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Contents and Storage

Storage Store at 2 to 8°C.

Contents

Reagents Provided	96 Test Kit	192 Test Kit
<i>Rt IL-18 Standard</i> , lyophilized, recombinant baculovirus Rt IL-18. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
<i>Standard Diluent Buffer</i> . Contains 8 mM sodium azide; 25 ml per bottle.	1 bottle	2 bottles
<i>Incubation Buffer</i> . Contains 8 mM sodium azide; 11 ml per bottle.	1 bottle	1 bottle
<i>Rt IL-18 High and Low Control</i> , lyophilized, recombinant baculovirus Rt IL-18. Refer to vial label for reconstitution volume and range.	2 vials	4 vials
<i>Rt IL-18 Antibody-Coated Wells</i> , 96 wells per plate.	1 plate	2 plates
<i>Rt IL-18 Biotin Conjugate</i> (Biotin-labeled anti-IL-18). Contains 8 mM sodium azide; 11 ml per bottle.	1 bottle	2 bottles
<i>Streptavidin-Peroxidase (HRP)</i> , (100x) concentrate. Contains 3.3 mM thymol; 0.125 ml per vial.	1 vial	2 vials
<i>Streptavidin-Peroxidase (HRP) Diluent</i> . Contains 3.3 mM thymol; 25 ml per bottle.	1 bottle	1 bottle
<i>Wash Buffer Concentrate (25X)</i> ; 100 mL per bottle.	1 bottle	1 bottle
<i>Stabilized Chromogen, Tetramethylbenzidine (TMB)</i> ; 25 mL per bottle.	1 bottle	1 bottle
<i>Stop Solution</i> ; 25 mL per bottle.	1 bottle	1 bottle
<i>Plate Covers</i> , adhesive strips.	4	6

Disposal Note

This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Purpose

The Invitrogen Rat Interleukin-18 (Rt IL-18) ELISA is to be used for the quantitative determination of IL-18 in rat serum, EDTA plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Rt IL-18.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Invitrogen Rt IL-18 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A polyclonal antibody specific for Rt IL-18 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Rt IL-18 content, control specimens, and unknowns, are pipetted into these wells.

During the first incubation, the Rt IL-18 antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated monoclonal antibody specific for Rt IL-18 is added. During the second incubation, this antibody binds to the immobilized Rt IL-18 captured during the first incubation.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Rt IL-18 present in the original specimen.

Background Information

IL-18, also known as Interferon-gamma Inducing Factor (IGIF), is a cytokine with Mr=18 kDa (157 amino acid residues) produced by macrophages and monocytes, Kupffer cells, keratinocytes, intestinal epithelial cells, osteoblasts, mouse diencephalon, and adrenal cortical cells of reserpine-treated rats. IL-18 is synthesized as an inactive precursor molecule with Mr=24 kDa which lacks a signal peptide. The IL-18 precursor is cleaved by IL-1 converting enzyme (ICE, Caspase-1), producing the bioactive, mature form. Only the mature, 18 kDa, form of IL-18 is secreted. Cells that respond to IL-18 include Th1-type cells and NK cells.

IL-18 exerts several effects on Th1-like cells. IL-18 stimulates Th1 cell proliferation, Fas ligand expression and IL-2R alpha chain expression.

IL-18 also works in combination with IL-12 to induce the production of IFN- γ , GM-CSF, and IL-2 by Th1-type cells. Standard bioassays for mIL-18 measure dose dependent IFN- γ production by IL-18 target cells, such as mouse IL-18 receptor transfected KG-1 cells (human myelomonocyte: ATCC CCL246). Immunomodulatory pathways, which include IL-18 stimulation of IFN- γ production, are under investigation. IFN- γ production by Th1-type cells and NK cells is important in many immune functions, including defense against viral and parasitic infections, enhancement of NK activity, activation of macrophages, enhancement of B cell function including B cell maturation, proliferation and immunoglobulin secretion, enhancement of MHC class I and class II antigen expression, and inhibition of osteoclast activation.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
 - Calibrated adjustable precision pipettes
 - Distilled or deionized water
 - Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
 - Glass or plastic tubes for diluting solutions
 - Absorbent paper towels
 - Calibrated beakers and graduated cylinders
-

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
 2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
 3. Samples should be collected in pyrogen/endotoxin-free tubes.
 4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
 5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
 6. It is recommended that all standards, controls and samples be run in duplicate.
 7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
 8. **Do not mix or interchange different reagent lots from various kit lots.**
 9. Do not use reagents after the kit expiration date.
 10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
 11. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
 12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. **Never** insert absorbent paper directly into the wells.
 13. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.
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Directions for Washing

- **Incomplete washing will adversely affect the test outcome.** All washing must be performed with the *Wash Buffer Concentrate (25X)* provided.
 - Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 ml of diluted *Wash Buffer*. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
 - Alternatively, the diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *Wash Buffer*, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
 - If using an automated washer, follow the washing instructions carefully.
-

Preparation of Reagents

Dilution of Standard

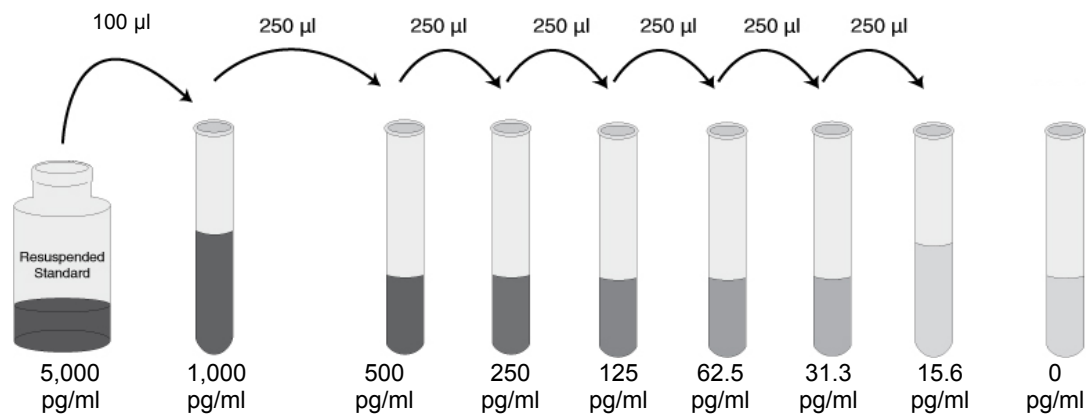
Note: Either glass or plastic tubes may be used for standard dilutions.

The Rt IL-18 standard was calibrated against a highly purified recombinant baculovirus protein.

1. Reconstitute standard to 5,000 pg/ml with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. It is recommended that standard be used within 1 hour of reconstitution.
2. Add 0.1 ml of the reconstituted standard to a tube containing 0.400 ml *Standard Diluent Buffer*. Label as 1,000 pg/ml Rt IL-18. Mix.
3. Add 0.250 ml of *Standard Diluent Buffer* to each of 6 tubes labeled 500, 250, 125, 62.5, 31.3 and 15.6 pg/ml Rt IL-18.
4. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.

Note

Remaining reconstituted standard should be discarded. Return the *Standard Diluent Buffer* to the refrigerator.



Preparing SAV-HRP

Note: Prepare within 15 minutes of usage. The *Streptavidin-HRP* (100x concentrate) is in 50% glycerol, which is viscous. To ensure accurate dilution, allow *Streptavidin-HRP* concentrate to reach room temperature. Gently mix. Pipette *Streptavidin-HRP* concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µl of this 100x concentrated solution with 1 ml of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.
2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 µl solution	2 ml
4	40 µl solution	4 ml
6	60 µl solution	6 ml
8	80 µl solution	8 ml
10	100 µl solution	10 ml
12	120 µl solution	12 ml

Dilution of Wash Buffer

1. Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 ml may be diluted up to 1.25 liters, 100 ml may be diluted up to 2.5 liters). Label as Working Wash Buffer.
 2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.
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Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 μ l of the *Standard Diluent Buffer* to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 μ l of standards, samples or controls to the appropriate microtiter wells. (See **Preparation of Reagents**.)
4. Add 50 μ l of *Incubation Buffer* to the zero standard wells and to the wells containing standards and serum/plasma samples, or 50 μ l of *Standard Diluent Buffer* to the wells containing cell culture samples and controls. Well(s) reserved for chromogen blank should be left empty. Tap gently on the side of the plate to mix.
5. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
7. Pipette 100 μ l of biotinylated *Rt IL-18 Biotin Conjugate* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
8. Cover plate with *plate cover* and incubate for **1 hour at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
10. Add 100 μ l Streptavidin-HRP Working Solution to each well except the chromogen blank(s). See **Preparation of Reagents**.
11. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
12. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
13. Add 100 μ l of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
14. Incubate for **30 minutes at room temperature and in the dark**. **Note: Do not cover the plate with aluminum foil or metalized mylar.** The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.

15. Add 100 μ l of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
16. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 μ l each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
17. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
18. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be diluted in *Standard Diluent Buffer* for serum/plasma samples or corresponding medium for cell culture samples and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

**Typical
Data
(Example)**

The following data were obtained for the various standards over the range of 0 to 1,000 pg/ml Rt IL-18.

Standard Rt IL-18 (pg/ml)	Optical Density (450 nm)
1,000	3.03
500	1.94
250	1.13
125	0.64
62.5	0.38
31.3	0.22
15.6	0.15
0	0.07

Performance Characteristics

Sensitivity The minimum detectable dose of Rt IL-18 is < 4 pg/ml. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Precision 1. Intra-Assay Precision
 Samples of known Rt IL-18 concentration were assayed in replicates of 22 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	93	387	877
SD	3.4	17.3	30.4
%CV	3.7	4.5	3.5
SD = Standard Deviation CV = Coefficient of Variation			

2. Inter-Assay Precision
 Samples were assayed 22 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	89	386	850
SD	6.0	17.1	38.4
%CV	6.7	4.4	4.5
SD = Standard Deviation CV = Coefficient of Variation			

Linearity of Dilution Rat serum and cell culture samples were serially diluted in *Standard Diluent Buffer* or RPMI containing 1% fetal bovine serum, respectively, over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.99.

Dilution	Serum			Cell Culture		
	Measured (pg/ml)	Expected (pg/ml)	% Expected	Measured (pg/ml)	Expected (pg/ml)	% Expected
neat	180	-		194	-	
1/2	87	90	97	90	97	93
1/4	43	45	96	44	48.5	91
1/8	28	22.5	124	25.7	24.3	105
1/16	14	11	124	13.5	12.1	111

Recovery The recovery of Rt IL-18 added to rat serum averaged 90%. The recovery of Rt IL-18 added to EDTA plasma averaged 95%. The recovery of Rt IL-18 added to tissue culture medium containing 1% fetal bovine serum averaged 101%, while the recovery of Rt IL-18 added to tissue culture medium containing 10% fetal bovine serum averaged 97%.

Specificity Buffered solutions of a panel of substances at 100 ng/ml were assayed with the Invitrogen Rt IL-18 kit. The following substances were tested and found to have no cross-reactivity: human IL-18, rat IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13, MIP-2, TNF- α , CINC-2 β , VEGF, GM-CSF; mouse IL-18. Both *E. Coli* and baculovirus derived Rt IL-18 were detectable with this kit.

Expected Values Four pools of rat serum and one pool of rat EDTA plasma were evaluated in this assay. The following concentrations were detected:

Sample	Concentrations
Pool serum 1	23 pg/ml
Pool serum 2	51 pg/ml
Pool serum 3	149 pg/ml
Pool serum 4	7 pg/ml
Pool EDTA plasma	6.5 pg/ml

Stimulation Protocols Cell culture supernatants were evaluated in this assay.

Rat Whole Blood (WB) cells were cultured in RPMI for 24, 48 or 72 hours either without stimulus or with a blend of LPS (25 mg/ml) and PHA (5 mg/ml), or with a blend of ionomycin (100 ng/ml) and PMA (100 ng/ml). Results are shown below.

Stimulus	Cell type	IL-18 (pg/ml)		
		24 hrs	48 hrs	72 hrs
None	WB cells	<4	<4	<4
LPS + PHA	WB cells	8	13	20
PMA + ionomycin	WB cells	8	22	27

Rat splenocytes were cultured at different cellular concentrations in RPMI supplemented with 5% FCS for 24, 48, 72 or 96 hours with a blend of LPS (25 mg/ml) and PHA (5 mg/ml). Results are shown below.

Cell concentration	Cell type	IL-18 (pg/ml)			
		24 hrs	48 hrs	72 hrs	96 hrs
0.25 x 10 ⁶ cells/ml	Splenocytes	17.4	27	36	45
0.8 x 10 ⁶ cells/ml	Splenocytes	49	ND	75	89
2.5 x 10 ⁶ cells/ml	Splenocytes	167	212	238	220

**Limitations
of the
Procedure**

Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with *Standard Diluent Buffer*, reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Rt IL-18 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Appendix

Troubleshooting Guide

Elevated background

Cause: Insufficient washing and/or draining of wells after washing. Solution containing either biotin or SAV-HRP can elevate the background if residual is left in the well.

Solution: Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid.

Cause: Contamination of substrate solution with metal ions or oxidizing reagents.

Solution: Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil.

Cause: Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate.

Solution: Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen.

Cause: Incubation time is too long or incubation temperature is too high.

Solution: Reduce incubation time and/or temperature.

Elevated sample/
standard
ODs

Cause: Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly.

Solution: Follow the protocol instructions regarding the dilution of the standard.

Cause: Incorrect dilution of the SAV-HRP conjugate.

Solution: Warm solution of SAV-HRP concentrate to room temperature, draw up slowly and wipe tip with kim-wipe to remove excess. Dilute ONLY in SAV diluent provided.

Cause: Incubation times extended.

Solution: Follow incubation times outlined in protocol.

Cause: Incubations carried out at 37°C when RT is dictated.

Solution: Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.

Poor
standard
curve

Cause: Improper preparation of standard stock solution.

Solution: Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample.

Cause: Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted.

Solution: NEVER substitute any components from another kit.

Cause: Errors in pipetting the standard or subsequent steps.

Solution: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no
color
develops

Cause: Reagents not at RT ($25 \pm 2^{\circ}\text{C}$) at start of assay.

Solution: Allow ALL reagents to warm to RT prior to commencing assay.

Cause: Incorrect storage of components, e.g., not stored at 2 to 8°C .

Solution: Store all components exactly as directed in protocol and on labels.

Cause: Working SAV-HRP solution made up longer than 15 minutes before use in assay.

Solution: Use the diluted SAV-HRP within 15 minutes of dilution.

Cause: TMB solution lost activity.

Solution 1: The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded.

Solution 2: Avoid contact of the TMB solution with items containing metal ions.

Cause: Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized.

Solution: Please contact Technical Support for advice when using nonvalidated sample types.

Cause: Wells have been scratched with pipette tip or washing tips.

Solution: Use caution when dispensing and aspirating into and out of microwells.

Poor
Precision

Cause: Errors in pipetting the standards, samples or subsequent steps.

Solution: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip.

Cause: Repetitive use of tips for several samples or different reagents.

Solution: Use fresh tips for each sample or reagent transfer.

Cause: Wells have been scratched with pipette tip or washing tips.

Solution: Use caution when dispensing and aspirating into and out of microwells.

Technical Support

Contact Us



For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

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For an up-to-date and complete list, visit www.invitrogen.com/ELISA or contact Technical Support.














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Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		<i>In vitro</i> diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
	Without, does not contain		With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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Rat IL-18 Assay Summary

