Quantikine® HS

Human IL-1β Immunoassay

Catalog Number HSLB50

For the quantitative determination of human interleukin 1 beta (IL-1β) concentrations in serum and plasma.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Interleukin 1 (IL-1) includes two distinct proteins, IL-1α and IL-1β, that play central roles in acute and chronic inflammation, both locally and systemically (1, 2). Human IL-1β is synthesized as a procytokine (269 amino acid) that is cleaved by IL-1β-converting enzyme to mature IL-1β (153 amino acid, 17 kDa) plus a prosegment (3, 4). Some combination of the mature form, the prosegment and pro-IL-1β is released from the cell.

IL-1β is produced primarily by monocytes and macrophages (5) but also by astrocytes, oligodendroglia, adrenal cortical cells, NK cells, endothelial cells, keratinocytes, megakaryocytes, platelets, neurons, neutrophils, osteoblasts, Schwann cells, trophoblasts, T cells, and fibroblasts (6 - 16). The most extensively studied function of IL-1 is initiation of inflammation. Bacterial endotoxin or a variety of non-microbial inflammatory substances induce production of IL-1, which is released into the local environment. IL-1 induces capillary endothelial cells to secrete chemokines (*e.g.* MCP-1) (17) and to increase expression of vascular adhesion molecules (*e.g.* E-selectin, ICAM-1 and VCAM-1) (18). MCP-1 activates mononuclear cell integrins (19), facilitating mononuclear infiltration into the area. With IL-12, IL-1 induces IFN-γ secretion by NK cells (20), resulting in an IFN-γ-induced activation of macrophages (21). Finally, IL-1 induces expression of matrix metalloproteinases (MMPs), leading to extracellular matrix degradation and monocyte migration, and to MMP-catalyzed degradation of IL-1β, a negative feedback (22). The effects of IL-1 are not, however, limited to inflammation. IL-1 is associated with bone formation and remodeling, insulin secretion, appetite regulation, fever induction, neuronal phenotype development, and IGF/GH physiology (23 - 28).

The action of IL-1 is mediated by IL-1 receptors. Many cells express two cell-surface IL-1 binding proteins (IL-1 RI and IL-1 RII) plus a non-binding receptor accessory protein (IL-1 RAcP) (29 - 34). Signaling is mediated by IL-1 RI together with IL-1 RAcP, which interacts only with IL-1 RI complexed with IL-1 (32 - 34). Signaling leads to activation of NF-κB-regulated transcription. IL-1 RII binds IL-1 but does not signal, therefore, it is considered a 'decoy', or down-regulating, receptor (35 - 37). There are soluble forms of IL-1 RI and IL-1 RII in serum (38, 39). Soluble IL-1 RI preferentially binds IL-1 receptor antagonist (IL-1ra) (38, 40), a non-signaling IL-1 family member that competes with IL-1 for receptor binding. By removing IL-1ra, soluble IL-1 RI enhances the action of IL-1 on cell-associated receptors. Soluble IL-1 RII differs from its membrane form in that its 2000-fold lower affinity for IL-1ra tends to enhance antagonist activity and its high affinity for the IL-1β precursor prevents extracellular processing to active IL-1β (41). Thus, IL-1 RII, either membrane-bound or soluble, suppresses the overall activity of IL-1.

Current methods for the assay of IL-1β are based on either the dose-dependent stimulation of ^{3}H-thymidine incorporation into PHA-stimulated thymocytes (LAF assay) (42) or proliferation of the murine T-helper cell line D10.G4.1 (43). These assays are tedious and do not distinguish between IL-1α and IL-1β. The Quantikine HS IL-1β Immunoassay is a solid phase ELISA that specifically measures human IL-1β in 18.5 - 24.5 hours (with an overnight incubation step). This kit is designed to measure IL-1β levels in serum and plasma. With the increased sensitivity of this kit, it is possible to measure the levels of IL-1β present in approximately 75% of serum samples from normal subjects.

The Quantikine HS IL-1β Immunoassay contains *E. coli*-expressed recombinant human IL-1β and antibodies raised against recombinant human IL-1β, and has been shown to accurately quantitate the recombinant factor. Results obtained using natural IL-1β showed linear curves that were parallel to the standard curves obtained using the Quantikine HS kit standards. These results indicate that the Quantikine HS Immunoassay kit can be used to determine relative mass values for natural IL-1β. Reports indicate that ELISA kits calibrated using mature IL-1β as a standard will detect, but considerably underestimate, the unprocessed IL-1β precursor present in samples (44, 45). In biological samples other than cell lysates, the precursor form of IL-1β is usually not the predominant form of IL-1β present and, additionally, is not biologically active. Therefore, results obtained using the Quantikine HS Immunoassay kit should provide a useful measure of the levels of active IL-1β present in serum and plasma.

PRINCIPLE OF THE ASSAY

DUE TO THE HIGH SENSITIVITY OF THIS KIT, PLEASE NOTE THE FOLLOWING PRECAUTIONS.

- Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.
- Use separate pipettes and glassware for dispensing all reagents to avoid cross-contamination.
- Careful washing of the microplate is essential to minimize nonspecific binding of the conjugate.
- IL-1β is detectable in saliva and sweat. Take precautionary measures to prevent contamination of the kit reagents while running the assay.

ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-1β is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier solution is added to the wells and color develops in proportion to the amount of IL-1β bound in the initial step. The color development is stopped and the intensity of the color is measured.

AMPLIFICATION SYSTEM

The Quantikine HS Immunoassay kit uses an amplification system in which the alkaline phosphatase reaction provides a cofactor that activates a redox cycle leading to the formation of a colored product (46 - 48). In this amplification system, alkaline phosphatase dephosphorylates the reduced form of nicotinamide adenine dinucleotide phosphate, NADPH (Substrate), to reduced nicotinamide adenine dinucleotide, NADH. The NADH subsequently serves as a specific cofactor that activates a redox cycle driven by the secondary enzyme system consisting of alcohol dehydrogenase and diaphorase (Amplifier). In the reaction catalyzed by diaphorase, NADH reduces a tetrazolium salt (INT-violet or iodonitrotetrazolium violet) to produce an intensely colored formazan dye and NAD^+. NAD^+ in turn is reduced by ethanol, in an alcohol dehydrogenase-catalyzed reaction, to regenerate NADH which can then re-enter the redox cycle. The rate of reduction of the tetrazolium salt and thus the amount of colored product formed are directly proportional to the amount of IL-1β bound in the initial step.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Although this kit has been designed to eliminate serum matrix problems, there may exist some serum or plasma samples that give falsely elevated values when assayed neat. If samples generate values that are suspiciously high or higher than the highest standard, dilute the samples in Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

REAGENTS

IL-1β Microplate* (Part 890243) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against IL-1β.

IL-1β Conjugate* (Part 890244) - 21 mL of polyclonal antibody against IL-1β, conjugated to alkaline phosphatase with preservative.

IL-1β Standard* (Part 890143) - 40 pg of recombinant human IL-1β in a buffered protein base with preservative, lyophilized.

Assay Diluent RD1C (Part 895007) - 2 vials (6 mL/vial) of a buffered protein base with preservative.

Calibrator Diluent RD6 (Part 895012) - 21 mL of animal serum with preservative.

Wash Buffer Concentrate (Part 895188) - 100 mL of a 10-fold concentrated solution of buffered surfactant with preservative.

Substrate (Part 895077) - Lyophilized NADPH with stabilizers.

Substrate Diluent (Part 895078) - 7 mL of buffered solution with stabilizers.

Amplifier (Part 895075) - Lyophilized amplifier enzymes with stabilizers.

Amplifier Diluent (Part 895076) - 7 mL of buffered solution containing INT-violet with stabilizers.

Stop Solution (Part 895032) - 6 mL of 2 N sulfuric acid.

Plate Covers - 8 adhesive strips.

*This product is covered by one or more of the following U.S. patents: 4,766,069; 5,510,462; 5,681,933; 4,762,914; 5,474,899; 5,789,185; 5,484,887; 5,122,459; 5,001,057; 5,077,219; 5,286,847.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	Store for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1C	
	Calibrator Diluent RD6	
	Conjugate	
	Standard	Store for up to 1 month at ≤ -20° C **in a manual defrost freezer.*** Avoid repeated freeze-thaw cycles.
	Substrate Solution	
	Amplifier Solution	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm, preferably with dual wavelength correction (correction wavelength set at 650 nm or 690 nm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, squirt bottle, or manifold dispenser.
- 1000 mL graduated cylinder.

PRECAUTIONS

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.

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

This kit contains Thimerosal, a mercury containing compound. The total amount of mercury in this kit is 12.5 mg.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed samples are not suitable for measurement of human IL-1β with this assay. Heparin and citrate are not recommended for use as anticoagulants in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

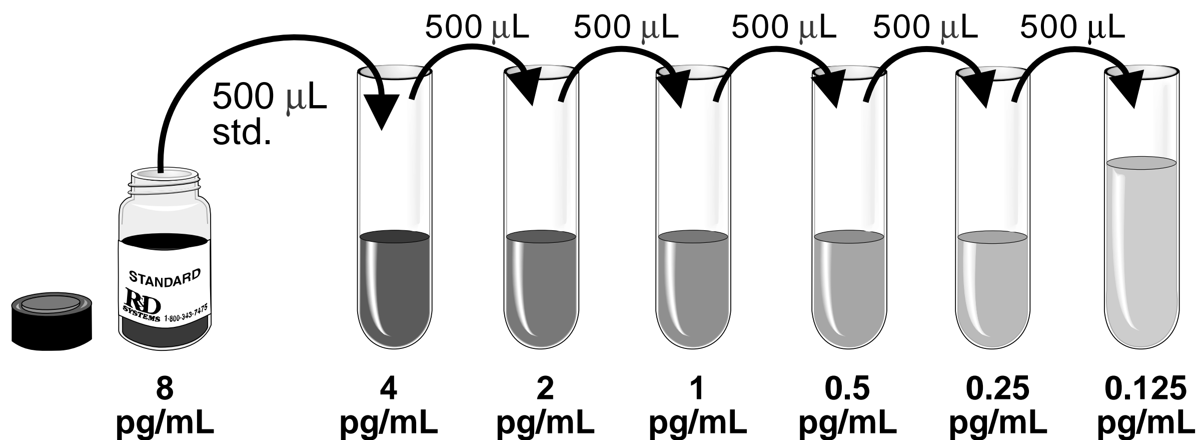
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Reconstitute the lyophilized Substrate in 6 mL of Substrate Diluent at least 10 minutes before use. Mix thoroughly. **Discard the rubber stopper after reconstitution**. Avoid contamination.

Amplifier Solution - Reconstitute the lyophilized Amplifier in 6 mL of Amplifier Diluent at least 10 minutes before use. Mix thoroughly. **Discard the rubber stopper after reconstitution**.

Standard - Reconstitute the IL-1β Standard with 5 mL of Calibrator Diluent RD6. This reconstitution produces a stock solution of 8 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 μL of Calibrator Diluent RD6 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (8 pg/mL). Calibrator Diluent RD6 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100 μL of Assay Diluent RD1C to each well.
4. Add 150 μL of Standard or sample per well. Cover with the adhesive strip provided. Incubate for 14 - 20 hours at room temperature.
5. Wash

   Notes on washing

   - *Excessive drying of the wells can lead to poor assay performance and imprecision. Subsequent reagents should be added immediately after washing the plate, and the wells not allowed to dry completely. Also avoid prolonged exposure of the wells to vacuum aspiration apparatus.*
   - *After removal of the adhesive strip prior to washing, the strip can be adhered to the underside of the wells to avoid the possibility of wells becoming dislodged during the decanting and rapping operations.*
   - *Inclusion of a 30 second soak between each addition of Wash Buffer and decanting the plate contents will improve the precision of the assay.*

   Wash Procedure

   a. Remove liquid from the wells by inverting the plate and decanting the contents.
   b. Remove excess liquid by grasping the plate firmly and smartly rapping the inverted plate on a clean paper towel at least 5 times.
   c. Fill each well with 400 μL of Wash Buffer using a squirt bottle, multi-channel pipette, or manifold dispenser.
   d. Remove liquid from the wells by inverting the plate and decanting the contents.
   e. Repeat steps b, c, and d three times for a total of 4 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.

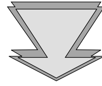
6. Add 200 μL of IL-1β Conjugate to each well. Cover with a new adhesive strip. Incubate for 3 hours at room temperature.
7. Repeat the wash as in step 5.
8. Add 50 μL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 45 minutes at room temperature. **Do not wash the plate**.
9. Add 50 μL of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 45 minutes at room temperature.

   Note: *Addition of Amplifier Solution initiates color development.*

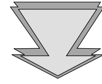
10. Add 50 μL of Stop Solution to each well. Addition of Stop Solution does not affect color in wells.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 nm without correction may be higher and less accurate.

ASSAY PROCEDURE SUMMARY

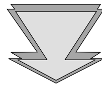
1. Prepare all reagents and standards as instructed.



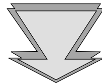
2. Add 100 μL Assay Diluent RD1C to each well.



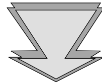
3. Add 150 μL Standard or sample to each well. Incubate 14-20 hrs. RT



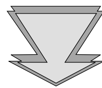
4. Wash 4 times.



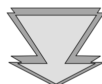
5. Add 200 μL Conjugate to each well. Incubate 3 hrs. RT



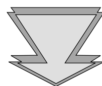
6. Wash 4 times.



7. Add 50 μL Substrate Solution to each well. Incubate 45 min. RT



8. Add 50 μL Amplifier Solution to each well. Incubate 45 min. RT



9. Add 50 μL Stop Solution to each well. Read at 490 nm within 30 min. λ correction 650 or 690 nm

CALCULATION OF RESULTS

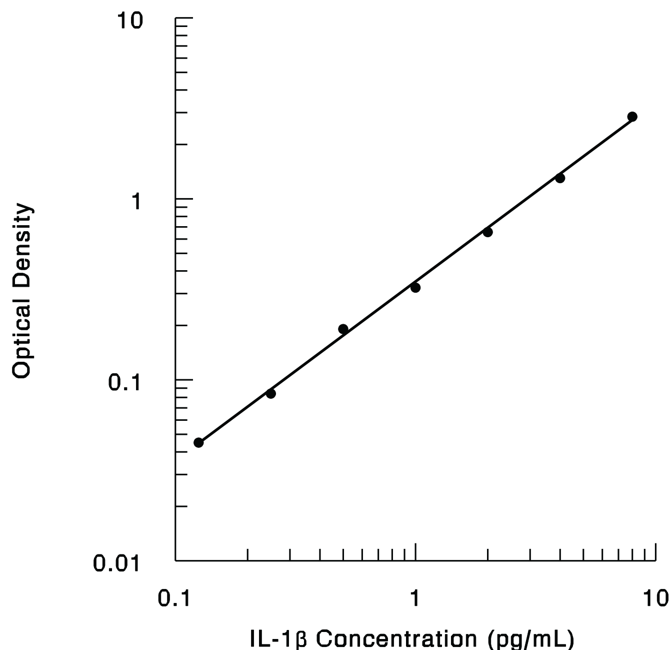
Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

To determine the IL-1β concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding IL-1β concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.172 0.184	0.178	-
0.125	0.215 0.231	0.223	0.045
0.25	0.261 0.262	0.262	0.084
0.5	0.364 0.374	0.369	0.191
1	0.507 0.495	0.501	0.323
2	0.849 0.817	0.833	0.655
4	1.504 1.458	1.481	1.303
8	3.026 3.020	3.023	2.845

TECHNICAL HINTS

- Neither the addition of the Substrate Solution nor the Stop Solution will result in a color change.
- Addition of the Amplifier Solution will result in a color change (colorless to shades of maroon).
- Amplifier Solution and Stop Solution should be added to the plate in the same order as the Substrate Solution.
- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	0.481	1.49	4.56	0.626	1.62	4.45
Standard deviation	0.049	0.103	0.291	0.120	0.167	0.365
CV (%)	10.2	6.9	6.4	19.2	10.3	8.2

RECOVERY

The recovery of IL-1β was determined by spiking to levels throughout the range of the assay in various matrices.

Sample Type	Average % Recovery	Range
Serum	93	84 - 108%
EDTA plasma	97	85 - 111%

LINEARITY

To assess linearity of the assay, the following biological samples, spiked with high concentrations of IL-1β, were diluted with Calibrator Diluent RD6 and then assayed.

		Serum	EDTA plasma
1:2	Average % Recovery	103	97
	Range (%)	95-113	87-111
1:4	Average % Recovery	97	101
	Range (%)	88-107	89-112
1:8	Average % Recovery	101	95
	Range (%)	85-114	86-113
1:16	Average % Recovery	92	93
	Range (%)	78-108	81-105

SENSITIVITY

The minimum detectable dose of IL-1β is typically less than 0.1 pg/mL.

The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-1β.

The NIBSC/WHO IL-1β 1st International Standard 86/680, which was intended as a potency standard, was evaluated in this kit. This standard is an *E. coli*-expressed recombinant human IL-1β.

The dose response curve of this 1st International Standard parallels the Quantikine HS standard curve. To convert sample values obtained with the Quantikine HS IL-1β kit to equivalent NIBSC 86/680 values, use the equation below.

NIBSC (86/680) equivalent value (IU/mL) = 0.1128 x Quantikine HS IL-1β value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Forty serum and plasma samples were evaluated in this assay.

Sample Type	Range (pg/mL)	% Detectable	Mean of Detectable (pg/mL)
Serum	ND - 1.996	75	0.536
EDTA plasma	ND - 1.633	45	0.514

ND = Non-detectable

SPECIFICITY

This assay recognizes both natural and recombinant human IL-1β. The factors listed below were prepared at 10 ng/mL in Calibrator Diluent RD6 and assayed for cross-reactivity. Preparations of the following factors at 10 ng/mL in a mid-range rhIL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Factors related to or associated with IL-1β:

rhIL-1α
rhIL-1ra
rhIL-1 sRI
rhIL-1 sRII
rmIL-1α
rmIL-1β

Other factors:

Recombinant human:
IL-2
IL-2 sRα
IL-3
IL-3 sRα
IL-4
IL-4 sR
IL-5
IL-5 sRα
IL-5 sRβ
IL-6
IL-6 sR
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13
ANG
AR
CNTF
ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
FGF-7
G-CSF
GM-CSF
sgp130
GROα
GROβ
GROγ
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
LAP
LIF
M-CSF
MCP-1
MIP-1α
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β3
TGF-β5
TGF-β sRII
TNF-α
TNF-β
sTNF RI
sTNF RII
VEGF

Recombinant mouse:
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
GM-CSF
TNF-α
SCF
LIF
MIP-1α
MIP-1β

Other:
bFGF acidic
bFGF basic
hPDGF
pPDGF
hTGF-β1
pTGF-β1
pTGF-β2

APPLICABLE PATENTS

These products are covered by the following patents:

Substrate (Part 895077) - Lyophilized NADPH with stabilizers.

Substrate Diluent (Part 895078) - 7 mL of buffered solution with stabilizers.

Amplifier (Part 895075) - Lyophilized amplifier enzymes with stabilizers.

Amplifier Diluent (Part 895076) - 7 mL of buffered solution containing INT-violet with stabilizers.

US: 4,446,231
4,595,655
4,598,042

EUROPE: 60,123
27,036

CANADA: 1,170,179

AUSTRALIA: 544,496

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