Sword ELISA Booster

For Human IL-1β

Catalog No. SB1B02-0500

For use with

Human IL-1β DuoSet from R&D Systems (Cat# DY201)

This package insert must be read before using this product.
For research use only.
Not for use in diagnostic procedures.

TABLE OF CONTENTS

Introduction	Page 3
Materials	Page 4
Storage and Handling	Page 4
Additional Materials Required	Page 5
Experimental Protocol	Page 4-7
Evaluation of Results	Page 7
Performance	Page 8-10
References	Page 10
Technical Support	Page 11
Important Licensing Information	Page 11
Appendix 1: Approved Detectors	Page 12
Appendix 2: Instrument Settings	Page 13-15

MANUFACTURED AND DISTRIBUTED BY:

Sword Diagnostics
3440 S. Dearborn Street, Suite 142S, Chicago, IL 60616
Telephone: (800)403-7075
info@sworddiagnostics.com

INTRODUCTION

Interleukin-1 is a pro-inflammatory cytokine that can be expressed in two separate isoforms, IL-1α and IL-1β.[1] IL-1β is constitutively expressed in an inactive pro-form that is activated through cleavage by Caspase-1.[2] IL-1β is expressed by dendritic cells and acts through the TNFα signaling pathway to induce expression of several inflammatory cytokines including IL-2, MIP-1β, and IFNγ.[3, 4] As a result, IL-1β has been identified as both a biomarker for inflammation diagnosis and a target for inflammation therapy. Current ELISAs lack the required sensitivity to quantify IL-1β in normal healthy donor serum for proper comparison with diseased individuals. At Sword Diagnostics, we have developed a reagent based on the principle of Raman Resonance that can easily be inserted into a standard IL-1β ELISA with greatly enhanced sensitivity. This ELISA Booster has been optimized for use with the R&D Systems DuoSet ELISA for Human IL-1B (Catalog No. DY201). This protocol allows the user to use 50 µL sample sizes instead of 100 µL as specified in the DuoSet literature.

MATERIALS

Kit Components

Part	Volume	Supplied
Sword ELISA Booster - Component A (10X)	**9 mL**	**1 - bottle**
Sword ELISA Booster - Component B (10X)	**9 mL**	**1 - bottle**
Sword Diluent - Component C (10X)	**9 mL**	**1 - bottle**
Sword Development Solution - Component D (5X)	**18 mL**	**1 - bottle**
Sword ELISA Blocker for IL-1β - Product no. SBL-502	**100 mL**	**1 - bottle**
Sword ELISA Assay Diluent for IL-1β – Product no.SDI-806	**100 mL**	**1 - bottle**
Sword ELISA HRP Conjugate for IL-1β – Product no. SCO-005	**25 µL**	**1 - vial**

Each Kit provides sufficient reagents for approximately 500 wells using a final reaction volume of 300 µL per assay. **Do not mix reagent components from different Reagent Kit lots.**

STORAGE AND HANDLING

Upon receipt, the kit should be stored at 2-8°C, protected from light. Stored properly, the kit components should remain stable until the expiration date designated with the kit. Allow reagents to warm to room temperature before opening component containers.

ADDITIONAL MATERIAL REQUIRED

Non-expired R&D Systems DuoSet for Human IL-1β (Cat# DY201)

Calibrator Diluent: PBS with 10% BSA, Freshly prepared

Coating Buffer: PBS

Wash Buffer: PBS with 0.5% Tween-20 freshly prepared

Deionized water

Proper pipettes and pipette tips

Multimode plate reader compatible with Sword ELISA Booster chemistry (see Appendix 1)

EXPERIMENTAL PROTOCOL

The following protocol is designed for use with the R&D Systems DuoSet for Human IL-1β (Cat# DY201). Qualified detectors are listed in Appendix 1.

Preparation of Working Solutions for Sword ELISA Booster

1.1 **Preparation of Sword ELISA Booster substrate solution:**

The following example is to prepare 16 mL Sword ELISA Booster substrate solution, enough for one 96 well plate. If you are not using a full plate, scale accordingly.

- Add the following to 11.2 mL deionized water:
- 1.6 mL Sword Booster - Component A (10X)
- 1.6 mL Sword Booster - Component B (10X)
- 1.6 mL Sword Diluent - Component C (10X)

Sufficient 10X Sword Diluent, Sword Booster Component A, and Sword Booster Component B have been provided to prepare sufficient Sword Booster to run 500 tests. This mixture is stable at 2-8° C for 3 days, but it is best if used within 1 - 3 hours. Protect this solution from air and light. The Sword substrate solution should be yellow in color.

Note: Salts in the Sword Diluent Component C (10X) may precipitate upon prolonged storage at 2-8°C. These salts readily re-dissolve by gentle inversion when the 10X solution is brought to Room Temperature. Check vial before using this reagent.

1.2 Preparation of 1X Sword Development Solution:

The following example is to prepare 16 mL Sword Development Solution, enough for one 96 well plate. If you are not using a full plate, scale accordingly.

- Add 3.2 mL 5X Sword Development Solution (Component D) to 12.8 mL deionized water.

Caution: *Both the 5X and 1X Development Solutions are caustic and should not come in contact with the skin*.

Assay Procedure

2.1 Reconstitute the Human IL-1β Capture Antibody with 0.5 mL of PBS to prepare a concentrate of mouse anti-human IL-1β antibody. Refer to the lot-specific Certificate of Analysis from R&D Systems for the amount supplied. Dilute the concentrated Human IL-1β Capture Antibody to a working concentration of 4 µg/ml in PBS, without carrier protein. Immediately coat a 96-well microplate with 100 µL/well of the diluted Human IL-1β Capture Antibody. Seal the plate and incubate overnight at 2-8°C.

2.2 Aspirate each well and wash a total of three times with 400 µl/well Wash Buffer (PBS + 0.5% Tween®20). Sufficient washing is essential for good assay performance. For each wash, allow Wash

Buffer to sit in plate for 15 – 30 s prior to aspiration. Remove liquid completely from each well between each wash. The use of an automated plate washer is highly recommended. After the final wash, remove any excess Wash Buffer by inverting the plate and blotting against clean paper towels.

2.3 Block plates by adding 200 µl Sword ELISA Blocker for IL-1β (SBL-502) to each well. Cover with a plate seal and incubate at room temperature for a minimum of 1 hour.

2.4 Repeat the aspiration/wash as in Step 2.2. The plates are now ready for sample addition.

2.5 Reconstitute Human IL-1β Standard with 0.5 mL of deionized or distilled water to prepare a concentrate of recombinant human IL-1β.

2.6 Prepare dilutions of standard and samples in calibrator diluent (PBS with 10% BSA). A seven-point standard curve using 3-fold serial dilutions in calibrator diluent, with a high standard of 100 pg/ml and a low standard of 0.046 pg/ml, is recommended. For serum and plasma samples, a high standard of 35 pg/ml and a low standard of 0.048 pg/ml is recommended.

2.7 Add 50 µl of Sword ELISA Assay Diluent for IL-1β (SDI-806) to each well. Then, add 50 µl standard or sample per well. Cover with a plate seal and incubate 2 hours at room temperature on a rotator/shaker.

2.8 Repeat the aspiration/wash as in Step 2.2 of Plate Preparation.

2.9 Reconstitute the Human IL-1β Detection Antibody with 1.0 mL calibrator diluent (10% BSA in PBS) to prepare a concentrate of biotinylated goat anti-human IL-1β. Refer to the lot-specific Certificate of Analysis from R&D Systems for the amount supplied. Dilute in calibrator diluent to the working concentration specified on the lot-specific Certificate of Analysis from R&D Systems.

2.10 Add 100 µl of the diluted Human IL-1β Detection Antibody to each well. Cover with a plate seal and

incubate 2 hours at room temperature on a rotator/shaker. **Avoid placing the plate in direct light.**

2.11 Repeat the aspiration/wash as in Step 2.2.

2.12 Prepare working concentration of Sword ELISA HRP Conjugate (SCO-005) by diluting 1.2 µL into 12 mL calibrator diluent (10% BSA in PBS) for a full 96 well plate.

2.13 Add 100 µl of the diluted Sword ELISA HRP Conjugate (SCO-005) to each well. Cover with a plate seal and incubate for 20 minutes at room temperature on a rotator/shaker. **Avoid placing the plate in direct light.**

2.14 Repeat the aspiration/wash as in Step 2.2.

2.15 Add 150 µL of the Sword ELISA Booster substrate solution (prepared in step 1.1) to each well. Incubate for 15 minutes at room temperature in the dark with no shaking.

2.16 Add 150 µL of Sword Development solution (prepared in step 1.2). The assay mixture should turn pink upon the addition of Sword Development solution. Incubate for 30 minutes in the dark. Due to the high volume in the wells, it is critical that caution is taken to avoid spilling contents. ***Do not put a lid on the plate. Do not shake.***

2.17 Determine the relative fluorescence units (RFU) of each well, using a microplate reader set to fluorescence with excitation and emission settings depending on the detector as listed in Appendix 1. Use the autocalculate function to determine the optimal gain setting. Signal will be stable for up to 90 minutes after development.

EVALUATION OF RESULTS

A standard curve is generated by plotting the mean signal values from the standard samples against the concentration of the standard samples.

3.1 For the most accurate results, the standard sample data should be fit to a four parameter logistic curve (4PLC) using the appropriate computer software for this iterative fitting process.

The 4PLC equation:

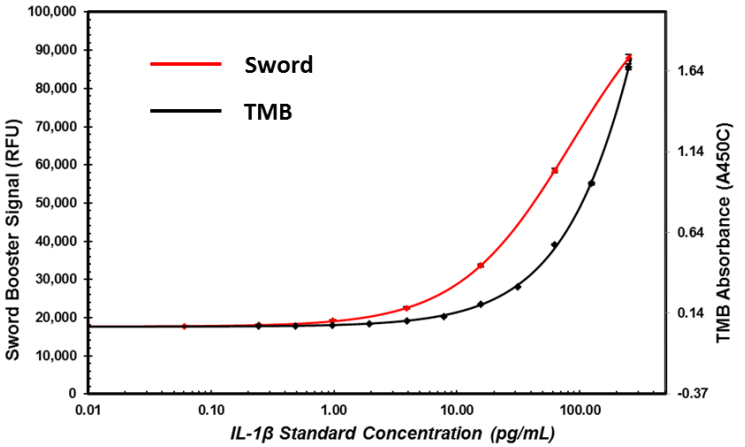
$$Y = D + \frac{(A-D)}{\left[1+\left(\frac{X}{C}\right)^{B}\right]}$$

communicated by D. Rodbard[5] has been used by Sword Diagnostics for this fitting process.

PERFORMANCE

Sensitivity

Figure 1. Sword ELISA Booster for IL-1β and TMB Curves



Human IL-1β standard curve (red line) generated as described in "Experimental Protocol" section of this document in comparison to TMB (black line). The curve was fit to a four parameter logistic curve (4PLC). Standard error bars are shown with each data point.

The **Limit of Detection** (LOD) of Human IL-1β with this procedure is typically **0.3 pg/mL** or less. The limit of detection, as defined by Sword Diagnostics, is the lowest concentration of standard with signal greater than the sum of the mean zero standard and the standard deviation of the zero standard values. The **Lower Limit of Quantitation** (LLOQ) is the lowest concentration of standard at or above the LOD, with back-calculated accuracy of 80 - 120% and %CV of 25% or less. The **Low Limit of Quantitation** (LLOQ) was **0.421 pg/mL**. The recommended assay range for this procedure is 0.42 - 100 pg/mL. The reported assay range for TMB in the R&D Systems DuoSet for Human IL-1β is 3.9 - 250 pg/mL with 100 µL sample size.[6]

Precision

Table 1: Precision of IL-1β Quantification in Human Serum with Sword ELISA Booster

	[IL-1β] Quantified (pg/mL)				
Donor	**Run 1**	**Run 2**	**Run 3**	**Average**	**CV%**
Very Low	1.07	0.79	0.76	0.87	19.6%
Low	1.38	1.08	1.09	1.18	14.4%
Medium	2.57	2.75	2.83	2.72	4.9%
High	4.05	3.34	3.70	3.70	9.6%

Table 1. IL-1β levels were quantified in human serum from healthy donors using the R&D Systems Human IL-1β DuoSet ELISA (DY201) with Sword ELISA Booster for Human IL-1β. Donor samples were tested in duplicate in three separate runs.

Table 2: Precision of IL-1β Quantification in Human Plasma EDTA with Sword ELISA Booster

	[IL-1β] Quantified (pg/mL)				
Donor	**Run 1**	**Run 2**	**Run 3**	**Average**	**CV%**
Low	0.35	0.35	0.51	0.40	22.9%
Medium	0.49	0.36	0.74	0.53	36.4%
High	0.68	0.57	0.44	0.56	21.3%

Table 2 IL-1β levels were quantified in human plasma EDTA from healthy donors using the R&D Systems Human IL-1β DuoSet ELISA (DY201) with Sword ELISA Booster for Human IL-1β. Donor samples were tested in duplicate in three separate runs.

Recovery

Table 3: Spike Recovery with Sword ELISA Booster for Human IL-1β in Serum

IL-1β Spike (pg/ml)	**Quantified IL-1β** (pg/ml)	**Accuracy %**	**CV%**
0.00	3.80	-	18.3%
0.05	3.30	86%	1.0%
0.14	4.06	103%	3.1%
0.43	4.86	115%	1.5%
1.30	6.66	131%	6.8%
3.89	9.11	119%	3.6%
11.67	16.55	107%	2.0%
35.00	39.18	101%	2.8%

Table 3. Human IL-1β Reference Standard was spiked into pooled human serum from healthy donors. Human IL-1β levels were quantified using the R&D Systems Human IL-1β DuoSet ELISA (DY201) with Sword ELISA Booster for Human IL-1β.

Table 4: Spike Recovery with Sword ELISA Booster for Human IL-1β in Plasma EDTA

IL-1β Spike (pg/ml)	Quantified IL-1β (pg/ml)	Accuracy %	CV%
0.00	0.46	-	32.0%
0.05	0.35	69%	54.5%
0.14	0.51	85%	19.5%
0.43	0.97	109%	14.4%
1.30	1.77	101%	6.2%
3.89	4.38	101%	1.5%
11.67	10.59	87%	2.1%
35.00	30.91	87%	4.0%

Table 4. Human IL-1β Reference Standard was spiked into pooled human plasma EDTA from healthy donors. Human IL-1β levels were quantified using the R&D Systems Human IL-1β DuoSet ELISA (DY201) with Sword ELISA Booster for Human IL-1β.

Linearity

Table 5: Linearity with Sword ELISA Booster for Human IL-1β in Diluted Serum and Plasma EDTA

	[IL-1β] Quantified (pg/mL)	
Dilution	**Serum**	**Plasma**
1:1	40.20	40.50
1:2	20.12	19.10
1:4	9.08	9.91

Table 5. Human IL-1β Reference Standard was spiked into pooled human serum and plasma EDTA from healthy donors. Spiked samples were diluted in calibrator diluent. Human IL-1β levels were quantified using the R&D Systems Human IL-1β DuoSet ELISA (DY201) with Sword ELISA Booster for Human IL-1β.

Quantification

Table 6: Quantification of IL-1β in Healthy Human Serum

	[IL-1β] Quantified (pg/mL)	
Donor	**Range**	**Average**
1	1.18 - 2.75	1.89
2	2.45 - 2.82	2.68
3	0.61 - 1.50	1.10
4	3.34 - 4.05	3.70
5	1.65 - 1.97	1.86
6	2.96 - 3.30	3.09
7	0.71 - 1.07	0.86
8	0.21 - 1.54	0.97
9	1.52 - 4.23	1.98
10	0.79 - 1.70	1.20
11	0.81 - 1.38	1.07
12	1.08 - 1.75	1.30

Table 6. Human IL-1β was quantified in human serum from twelve healthy donors using the R&D Systems Human IL-1β DuoSet ELISA (DY201) with Sword ELISA Booster for Human IL-1β.

Table 7: Quantification of IL-1β in Healthy Human Plasma

	[IL-1β] Quantified (pg/mL)	
Donor	**Range**	**Average**
1	0.37 - 0.47	0.44
2	ND	ND
3	0.369 - 0.50	0.42
4	ND	ND
5	0.36 - 0.50	0.44
6	ND	ND
7	ND	ND
8	ND	ND
9	ND	ND
10	0.03 - 0.41	0.15
11	0.03 - 0.86	0.37
12	0.48 - 0.73	0.64

Table 7. Human IL-1β was quantified in human plasma EDTA from twelve healthy donors using the R&D Systems Human IL-1β DuoSet ELISA (DY201) with Sword ELISA Booster for Human IL-1β.

REFERENCES

[1]Weber A, et al: Interleukin-1beta (IL-1beta) processing pathway. Sci Signal 2010, 3:cm2.
[2]Martinon et al: The inflammasomes: guardians of the body. Annual review of immunology 2009, 27:229-65.
[3]Luft T, et al: IL-1 beta enhances CD40 ligand-mediated cytokine secretion by human dendritic cells (DC): a mechanism for T cell-independent DC activation. Journal of immunology 2002, 168:713-22.
[4]Zhang et al: Interleukin-1beta induces macrophage inflammatory protein-1beta expression in human hepatocytes. Cell Immunol 2003, 226:45-53.
[5]Rodbard D: Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Clinical chemistry 1974, 20:1255-70.
[6]www.rndsystems.com. R&D Systems Quantikine ELISA for Human IL-33, 2015.

TECHNICAL SUPPORT

For any questions or technical support regarding this product, please contact:

Sword Diagnostics
3440 S. Dearborn Street, Suite 142S, Chicago, IL 60616
Telephone: (800)403-7075
techsupport@sworddiagnostics.com

IMPORTANT LICENSING INFORMATION

This product may be covered by one or more Limited Use Label Licenses (see the Label License on the Product Packaging or our website, www.SwordDx.com). By use of this product you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, this product is for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

APPENDIX 1. COMPATIBLE DETECTORS

Vendor	Model	Sword Compatible	Filter Mode	Mono Chrometer	Red PMT
BioTek	Cytation 5	Yes	Best	Ok	Best
BioTek	Cytation 3	Yes	Good	Ok	Best
BioTek	Synergy H1, H4, 2	Yes	Good	Ok	Best
BioTek	NEO, NEO2	Yes	Best	Good	Best
Tecan	Infinite M1000 Pro	Yes	n/a	Best	n/a
Tecan	Infinite F500 Pro	Yes	Best	n/a	n/a
Tecan	Infinite 200 Pro	Yes	n/a	Good	n/a
Tecan	Spark 10m / 20M	Yes	n/a	Best	n/a
Molecular Devices	SpectroMax Paradigm	Yes	Good	Ok	
Molecular Devices	SpectraMax M Series	No			
Perkin Elmer	Envision	No			
Perkin Elmer	Victor	No			

APPENDIX 2. INSTRUMENT SETTINGS

BioTek Cytation 5 Parameter	Setting
Detection Method	Flourescence Intensity
Read Type	Endpoint/Kinetic
Optics Type	Filter
Excitation Wavelength	530 nm
Excitation Bandwidth	25 nm
Emission Wave Length	730 nm
Emission Bandwidth	40 nm
Optics Position	Top 570 nm
Gain	Extended
Read Height	Calibrate for high well (usually A1)

Note: A red-shifted PMT is recommended for best results, but not critical.

BioTek Synergy H4 Parameter	Setting
Detection Method	Fluorescence Intensity
Read Type	Endpoint/Kinetic
Optics Type	Filter
Excitation Wavelength	530 nm
Excitation Bandwidth	25 nm
Emission Wave Length	680 nm*
Emission Bandwidth	30 nm
Optics Position	570
Gain[a]	Extended
Read Height	Calibrate for high well (usually A1)

*Note: *The 680 nm emission filter is recommended for in the struments without a red-shifted PMT. If the H4 being used has a red-shifted PMT, we recommend the 730 nm/40 nm BW emission filter.*

BioTek NEO2 Parameter	Setting
Detection Method	Flourescence Intensity
Read Type	Endpoint/Kinetic
Optics Type	Filter
Excitation Wavelength	530 nm
Excitation Bandwidth	25 nm
Emission Wave Length	730 nm
Emission Bandwidth	40 nm
Optics Position	Top 570 nm
Gain	Autoscale to high well (usually A1)
Read Height	Calibrate for high well (usually A1)

Note: The NEO2 is equipped with the red-shifted PMT, and it should be used for our application.

Tecan Infinite M1000 Pro Parameter	Setting
Measurements	Fluorecent Intensity
Mode	Top
Excitation Wavelength	530 nm
Excitation Bandwidth	20 nm
Emission Wave Length	700 nm
Emission Bandwidth	20 nm
Flashes	25 (100 Hz)
Integration	20 µsec
Lag Time	0 µsec
Gain[a]	Auto based on high well (usually A1)
Z-Position	Auto based on high well (usually A1)