Sword ELISA Booster

For Human IL-33

Catalog No. SB-HIL3301-05

For use with

Human IL-33 Quantikine® ELISA from R&D® Systems (Cat# D3300)

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

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

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INTRODUCTION

IL-33 is a nuclear cytokine that is part of the innate immune response mechanism, and is frequently linked to inflammation in diseases such as asthma, Crohn's disease, and atopic dermatitis. Interaction between IL-33 and the ST2-1RAcP receptor[1] activates signaling pathways in several innate immune lymphoid cells, including natural killer, Th2, basophils, and eosinophils as a means of clearing out invading microorganisms.[2,3] IL-33 is constitutively expressed in the cell nuclei of normal healthy individuals.[4] Damage to the cell causes the release of IL-33 into the extracellular matrix which then signals the beginning of the inflammatory process.[5] As a result, IL-33 functions as an alarmin and subsequently has been identified as a strong biomarker candidate for inflammation. This Sword ELISA Booster has been optimized for use with the R&D Systems Quantikine ELISA for Human IL-33 (Cat#D3300).

MATERIALS

Booster 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**

Each Booster 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 Booster lots.**

STORAGE AND HANDLING

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

ADDITIONAL MATERIAL REQUIRED

R&D Systems Human IL-33 Quantikine ELISA Kit (Cat# D3300)

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 Quantikine ELISA for Human IL-33 (Cat# D3300). 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 IL-33 reference standard with amount of deionized water stated on the vial. This reconstitution produces a stock solution of 4000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

2.2 Dilute Calibrator Diluent (RD5-26) supplied with the R&D Systems Quantikine ELISA for Human IL-33 at a 1:5 ratio. Prepare serial dilutions of standards in diluted Calibrator diluent sufficient to generate standard curve with values below 1 pg/mL. Calibrator diluent alone serves as the zero standard (0 pg/mL). Also prepare samples for analysis.

2.3 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

2.4 Add 100 µL of Assay Diluent (RD1-77) supplied with the R&D Systems Quantikine ELISA for Human IL-33 to each well.

2.5 Add 100 µL of Standard, control, or sample to each well. Cover with the adhesive strip provided. Incubate overnight at 4°C with shaking.

2.6 Aspirate each well and wash with 1X 400 µL wash buffer supplied with the R&D Systems Quantikine ELISA for Human IL-33, repeating the process 5 times for a total of 6 washes. Complete removal of liquid at each step is essential to good performance. Use of an automated plate washer is recommended. After the last wash, remove any excess wash buffer by aspirating or decanting. Invert plate and blot it against clean paper towels.

2.7 Add 200 µL of IL-33 Conjugate to each well. Cover with a new adhesive strip. Incubate with shaking for 2 hours at room temperature.

2.8 Repeat the aspiration/wash as in step 2.6.

2.9 Add 150 µL of the Sword Booster solution (prepared in step 1.1) to each well. Incubate for 15 minutes at room temperature in the dark.

2.10 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.***

2.11 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 auto calculate 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[4] has been used by Sword Diagnostics for this fitting process.

PERFORMANCE

Sword Booster Performance Human IL-33		DuoSet® Insert	Sword Boost	Quantikine® Insert	Sword Boost
Assay Range:	0.52-100 pg/mL	23.4-1500 pg/mL	45.0x	6.25-400 pg/mL	12.0x
LLOQ:	1.5 pg/mL	23.4 pg/mL	15.6x	6.25 pg/mL	4.2x
Sample Size:	100 µL	100 µL		100 µL	
Spike Recovery: (Plasma)	3.1 pg/mL				
Spike Recovery: (Serum)	3.1 pg/mL				

Sensitivity

Figure 1. Sword ELISA Booster for IL-33

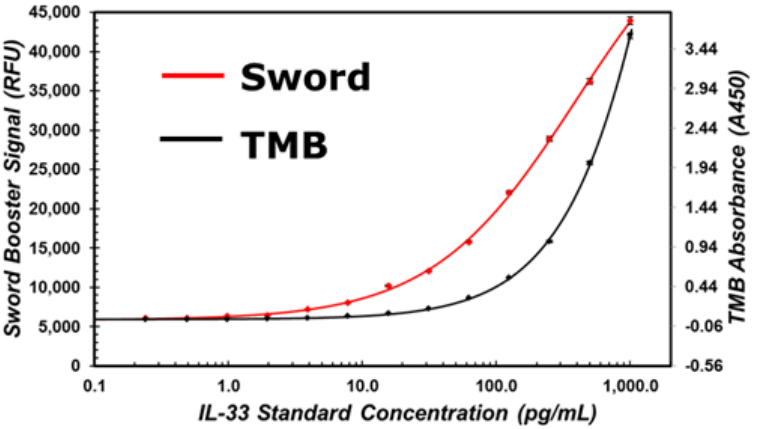

Figure 1. Human IL-33 standard curve (red line) generated as described in “Experimental Protocol” section of this document in comparison to TMB (black line). The curve was fit to a four parameter logistic curve (4PLC). Standard error bars are shown with each data point.

The **Limit of Detection** (LOD) of Human IL-33 with this procedure is **0.52 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 LLOQ with this procedure is **1.5 pg/mL.** The recommended assay range for this procedure is 0.52 – 100 pg/mL.

The sensitivity reported for TMB in the R&D Systems Quantikine ELISA for Human IL-33 is 1.65 pg/mL, and the reported assay range for TMB is 6.25 - 400 pg/mL.[7]

Precision

Table 1: Precision of IL-33 Quantification in Human Serum with Sword ELISA Booster

	[IL-33] Quantified (pg/ml)				
Donor	**Run 1**	**Run 2**	**Run 3**	**Average**	**CV%**
Very Low	0.25	0.53	0.50	0.43	36.7%
Low	1.37	1.13	1.20	1.23	10.0%
Medium	5.68	4.79	4.72	5.06	10.6%
High	87.67	82.86	84.40	84.98	2.9%

Table 1. IL-33 levels were quantified in human serum from healthy donors using the R&D Systems Human IL-33 Quantikine ELISA (D3300) with Sword ELISA Booster for Human IL-33. Donor samples were tested in duplicate in three separate runs.

Table 2: Precision of IL-33 Quantification in Human Plasma EDTA with Sword ELISA Booster

	[IL-33] Quantified (pg/ml)				
Donor	**Run 1**	**Run 2**	**Run 3**	**Average**	**CV%**
Very Low	0.90	0.66	0.19	0.58	62.2%
Low	0.96	0.92	1.31	1.06	20.2%
Medium	5.25	4.57	4.41	4.74	9.4%
High	7.56	7.19	9.03	7.93	12.2%

Table 2. IL-33 levels were quantified in human plasma EDTA from healthy donors using the R&D Systems Human IL-33 Quantikine ELISA (D3300) with Sword ELISA Booster for Human IL-33. Donor samples were tested in duplicate in three separate runs.

Recovery

Table 3: Spike Recovery with Sword ELISA Booster for Human IL-33 in Serum

IL-33 Spike (pg/ml)	Quantified IL-33 (pg/ml)	Accuracy %	CV%
0.00	1.50	-	21.2%
1.56	3.87	127%	14.7%
3.13	5.13	111%	0.1%
6.25	8.56	111%	8.9%
12.50	14.82	106%	5.1%
25.00	26.48	100%	6.2%
50.00	52.78	102%	7.0%
100.00	105.38	104%	5.3%

Table 3. Human IL-33 Reference Standard was spiked into pooled human serum from healthy donors. Human IL-33 levels were quantified using the R&D Systems Human IL-33 Quantikine ELISA (D3300) with Sword ELISA Booster for Human IL-33.

Table 4: Spike Recovery with Sword ELISA Booster for Human IL-33 in Plasma EDTA

IL-33 Spike (pg/ml)	Quantified IL-33 (pg/ml)	Accuracy %	CV%
0.00	1.30	-	20.3%
1.56	3.65	127%	20.9%
3.13	4.90	111%	12.2%
6.25	8.06	107%	9.9%
12.50	14.22	103%	5.2%
25.00	26.61	101%	5.5%
50.00	54.08	105%	5.2%
100.00	106.29	105%	2.5%

Table 4. Human IL-33 Reference Standard was spiked into pooled human plasma EDTA from healthy donors. Human IL-33 levels were quantified using the R&D Systems Human IL-33 Quantikine ELISA (D3300) with Sword ELISA Booster for Human IL-33.

Linearity

Table 5: Linearity with Sword ELISA Booster for Human IL-33 in Diluted Serum and Plasma EDTA

	[IL-33] Quantified (pg/mL)	
Dilution	Serum	Plasma
1:1	109.13	107.30
1:2	50.14	49.85
1:4	25.85	24.96

Table 5. Human IL-33 Reference Standard was spiked into pooled human serum and plasma EDTA from healthy donors. Spiked samples were diluted in 1:5 Calibrator Diluent RD5-26. Human IL-33 levels were quantified using the R&D Systems Human IL-33 Quantikine ELISA (D3300) with Sword ELISA Booster for Human IL-33.

Quantification

Table 6: Quantification of IL-33 in Healthy Human Serum

	[IL-33] Quantified (pg/mL)	
Donor	**Range**	**Average**
1	0.15 - 0.72	0.53
2	0.25 - 0.53	0.43
3	4.72 - 5.68	5.06
4	0.27 - 0.53	0.36
5	3.64 - 5.38	4.68
6	0.32 - 0.72	0.52
7	1.26 - 3.04	2.36
8	0.76 - 1.13	0.91
9	0.64 - 1.43	0.92
10	1.41 - 1.76	1.57
11	1.13 - 1.37	1.23
12	82.86 - 87.67	84.98

Table 6. Human IL-33 was quantified in human serum from twelve healthy donors using the R&D Systems Human IL-33 Quantikine ELISA (D3300) with Sword ELISA Booster for Human IL-33. Measured human IL-33 levels varied from 0.15 pg/ml to 87.67 pg/ml. Mean and median human IL-33 levels were 8.63 pg/ml and 1.08 pg/ml, respectively.

Table 7: Quantification of IL-33 in Healthy Human Plasma

	[IL-33] Quantified (pg/mL)	
Donor	**Range**	**Average**
1	0.91 - 1.38	1.11
2	4.41 - 5.25	4.74
3	0.19 - 0.90	0.58
4	0.92 - 1.31	1.06
5	7.19 - 9.03	7.93
6	0.20 - 1.75	0.99
7	1.17 - 2.97	2.11
8	0.24 - 1.12	0.67
9	1.22 - 3.49	2.32
10	1.35 - 3.41	2.42
11	1.07 - 2.26	1.74
12	1.18 - 2.04	1.54

Table 7. Human IL-33 was quantified in human plasma EDTA from twelve healthy donors using the R&D Systems Human IL-33 Quantikine ELISA (D3300) with Sword ELISA Booster for Human IL-33. Measured human IL-33 levels varied from 0.19 pg/ml to 9.03 pg/ml. Mean and median human IL-33 levels were 2.27 pg/ml and 1.64 pg/ml, respectively.

REFERENCES

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TECHNICAL SUPPORT

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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	Fluorescence 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 instruments 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	Fluorescence 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	Fluorescence 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)