MSD® MULTI-ARRAY Assay System

Mouse IL-22 Kit

1-Plate Kit	K152SFD-1
5-Plate Kit	K152SFD-2
25-Plate Kit	K152SFD-4

MSD Cytokine Assays

Mouse IL-22 Kits

For use with 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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Ordering Information

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Introduction

Interleukin-22 (IL-22), first described as IL-10-related T cell inducible factor, is part of the IL-10 superfamily notable for its pro-inflammatory or anti-inflammatory actions, which are dependent on the context of its expression.[1-3] It is produced by Th17 cells and NK cells, and especially in epithelial cells like those in the skin, lung, liver, and intestine.[1,4] IL-22 binds heterodimer IL-22R that is made up of IL-22R1 and IL-10R2.[1-3]

IL-22 concentration in synovial fluid and serum is higher in rheumatoid arthritis patients than in osteoarthritic or normal patients.[3] This is thought to be the result of the induction of RANKL and increased osteoclast production leading to joint inflammation.[3] In contrast, IL-22 plays a protective and anti-inflammatory role in alcohol liver injury with increased levels of IL-22 reflecting reduced markers of oxidative stress, inflammation, and injury in serum.[4] Improved liver health is corroborated through histological examination.[4] In another example of anti-inflammatory properties, IL-22 is upregulated in allergic airway inflammation to attenuate the inflammatory activity of eosinophils and possibly the subsequent production of pro-inflammatory cytokines and chemokines.[2]

Principle of the Assay

MSD cytokine assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Mouse IL-22 is a sandwich immunoassay. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots in the layout shown below. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

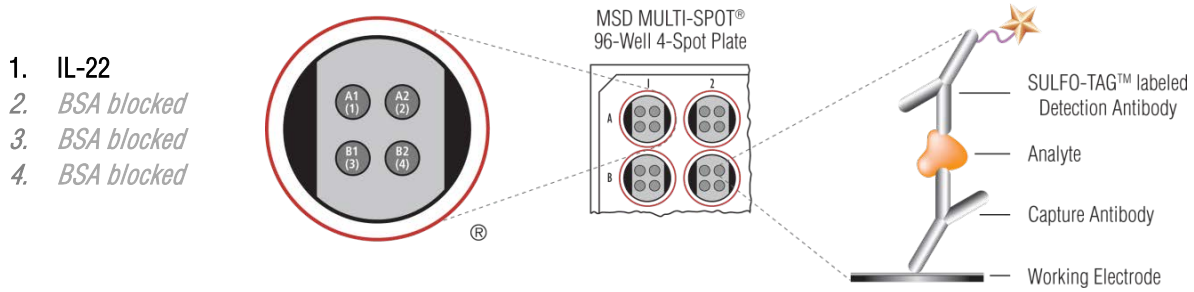


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Reagents Supplied

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1 Plate Kit	5 Plate Kit	25 Plate Kit	
MULTI-SPOT® 96-well, 4-Spot Mouse IL-22 Plate	2–8°C	N452SFA-1	4-Spot	1	5	25	96-well plate, foil sealed with desiccant.
SULFO-TAG Anti-ms IL-22 Antibody (50X)	2–8°C	D22SF-2	75 µL	1			SULFO-TAG–conjugated antibody
		D22SF-3	375 µL		1	5	
Mouse IL-22 Calibrator (200 ng/mL)	≤-70°C	C02SF-2	60 µL	1 vial	5 vials	25 vials	Recombinant mouse IL-22 protein in a buffered protein diluent.
Diluent 41	≤-10°C	R50AH-2	50 mL	1 bottle	1 bottle	5 bottles	Diluent for samples and calibrator; contains serum, blockers, and preservatives.
Diluent 45	≤-10°C	R50AI-2	25 mL	1 bottle	1 bottle	5 bottles	Diluent for detection antibody; contains protein, blockers, and preservatives.
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles	MSD buffer to catalyze the electrochemiluminescence reaction

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing serial dilutions
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 300–1000 rpm.
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 for plate washing or MSD Wash Buffer, catalog # R61AA-1
- ❑ Adhesive plate seals
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the material safety data sheet (MSDS), which can be obtained from MSD Customer Service.

Best Practices and Technical Hints

- Do not mix or substitute reagents from different sources or different kit lots.
- Thaw diluent bottles in a water bath at room temperature to ensure uniform thawing.
- Dilute calibrators, samples, and controls in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Shaking should be vigorous with a rotary motion between 300 and 1000 rpm.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid introduction of bubbles, and pipette to the bottom corner of an empty well.
- When using an automated plate washer, rotating the plate 180 degrees between wash steps may improve assay precision.
- Gently tap the plate to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keeping time intervals consistent between adding read buffer and reading the plate should improve inter-plate precision. Limit the amount of time the plate is incubated with read buffer.
- No shaking is necessary after adding read buffer.
- Remove plate seals prior to reading the plate.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the plate.
- If assay results are above the top of the calibration curve, dilute samples, and repeat the assay.
- When running a partial plate, seal the unused sectors (see sector map in instrument and software manuals) to avoid contaminating unused wells. (Remove all seals before reading.) Partially used plates may be sealed and stored up to 30 days at 2–8°C in the original foil pouch with desiccant.
- You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature.

> **Important:** Upon first thaw, separate diluents into aliquots appropriate for the size of your needs before refreezing.

Prepare Calibrator Dilutions

MSD supplies calibrator for the Mouse IL-22 Kit at 20-fold higher concentration than the recommended highest calibrator. We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the calibration curve solutions.

Calibrator	Mouse IL-22 (pg/mL)	Dilution Factor
Stock Calibrator	200 000	
Calibrator-01	10 000	20
Calibrator-02	2500	4
Calibrator-03	625	4
Calibrator-04	156	4
Calibrator-05	39	4
Calibrator-06	9.8	4
Calibrator-07	2.4	4
Calibrator-08	0	n/a

To prepare 7 calibration solutions plus a zero calibrator blank for up to 4 replicates:

1. Prepare the highest calibrator by adding 20 µL of stock calibrator to 380 µL of Diluent 41. Mix well.
2. Prepare the next calibrator by transferring 100 µL of the highest calibrator to 300 µL of Diluent 41. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
3. Use Diluent 41 as the blank.

Sample Collection and Handling

Below are general guidelines for mouse sample collection, storage, and handling. If possible, use published guidelines.[5,6] Evaluate sample stability method as needed.

- When preparing serum, allow samples to clot for 2 hours at room temperature, then centrifuge for 20 minutes at 2000 g prior to use or freezing.
- Centrifuge plasma for 20 minutes at 2000 g prior to use or freezing.
- For samples other than serum and plasma, use immediately or freeze.

Freeze all samples in aliquots; they may be stored at ≤-10°C until needed. Repeated freeze–thaw of samples is not recommended. After thawing, centrifuge samples at 2000 g for 3 minutes to remove particulates prior to sample preparation.

Dilute Samples

Dilute samples with Diluent 41. For mouse serum and plasma samples, MSD recommends a minimum 2-fold dilution. For example, to dilute 2-fold, add 60 µL of sample to 60 µL of Diluent 41. You may conserve sample volume by using a higher dilution.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working detection antibody solution used in the assay is a 1X solution. Prepare the detection antibody solution immediately prior to use.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-ms IL-22 Antibody
- ❑ 2940 µL of Diluent 45

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 month.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates may be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

1. **Add Sample:** Add 50 µL of diluted sample, calibrator, or control per well. Seal the plate with an adhesive plate seal and incubate with shaking for 2 hours at room temperature.

2. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate with shaking for 2 hours at room temperature.

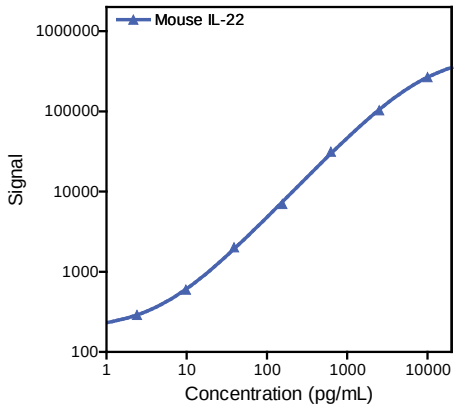
3. **Wash and Read:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Read the plate on the MSD instrument. No incubation in read buffer is required before reading the plate.

Curve Fitting

Run at least 1 set of calibrators in duplicate to generate the calibration curve. The calibration curve is modeled using least squares fitting algorithms so that signals from the calibrators can be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (4 logs), which allows for accurate quantification in samples without the need for multiple dilutions or repeated testing. The data displayed below were generated by DISCOVERY WORKBENCH® analysis software using a 4-parameter, logistic curve-fitting model (sigmoidal dose-response) with a $1/Y^2$ weighting function, which provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve.

Typical Data

The following calibration curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of 2 replicates of calibrators.



Mouse IL-22		
Conc. (pg/mL)	Average Signal	%CV
0	177	2.4
2.4	290	3.9
9.8	601	8.6
39	2017	15.8
156	6997	9.4
625	31 528	4.8
2500	103 125	1.0
10 000	267 452	1.2

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to a signal 2.5 standard deviations above the background (zero calibrator).

	Mouse IL-22
LLOD (pg/mL)	3.3

Assay Components

Calibrators

The assay calibrator uses recombinant mouse IL-22, (residues 34–179), expressed in *E. coli*.

Antibodies

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
IL-22	Goat Polyclonal	Goat Polyclonal	A

References

1. Sonnenberg G, et al. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nature*. 2011;12:383-90.
2. Hirose K, et al. Roles of IL-22 in allergic airway inflammation. J Allergy. 2013. doi:10.1155/2013/260518.
3. Kim KW, et al. Interleukin-22 promotes osteoclastogenesis in rheumatoid arthritis through induction of RANKL in human synovial fibroblasts. *Arthritis & Rheumatism*. 2012;64:1015-1023.
4. Ki SH, et al. Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. *Hep*. 2010;52:1291-1300.
5. Hem A, et al. Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, ferret and mink. Lab Anim. 1998;32:364-8.
6. Removal of blood from laboratory animals and birds: First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement. Lab Anim. 1993;27:1-22.

Summary Protocol

Mouse IL-22 Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Mouse IL-22 assays.

Sample and Reagent Preparation

Bring all reagents to room temperature.

Prepare 7 calibration solutions using the supplied calibrator:

- Dilute the stock calibrator 20-fold in Diluent 41.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator.

Dilute samples and controls 2-fold in Diluent 41 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 45.

Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Sample

Add 50 µL/well of sample (calibrators, controls, or unknowns).

Incubate at room temperature with shaking for 2 hours.

Step 2: Wash and Add Detection Antibody Solution

Wash plate 3 times with at least 150 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with shaking for 2 hours.

Step 3: Wash and Read Plate

Wash plate 3 times with at least 150 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Read plate on MSD imager.

Plate Diagram