Millipore® SigmaAldrich.com

User Guide

SMC® 2-Plex Human IL-23, IL-22 High Sensitivity Immunoassay Kit

Microparticle Assay

Human IL-23, IL-22 Immunoassay Kit for the Quantitative Determination of IL-23 and IL-22 in Human Serum and Plasma

03-0215-2P

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Introduction

Interleukin-23 (IL-23) and Interleukin-22 (IL-22) both play significant roles in the progression of various autoimmune and inflammatory diseases through their effects on immune responses. IL-23 primarily drives the inflammatory response through its role in promoting Th17 cells and their cytokines, while IL-22 contributes to inflammation and tissue damage in various autoimmune diseases. Both IL-23 and IL-22 are involved in the pathogenesis of chronic inflammatory conditions, such as inflammatory bowel disease (ulcerative colitis and Crohn’s disease), rheumatoid arthritis, psoriasis, and multiple sclerosis, making them important targets for therapeutic interventions.

The SMC® Human IL-23, IL-22 High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure IL-23 and IL-22 in Human Serum and Plasma. Capture antibodies specific for human IL-23 and IL-22 have been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the IL-23 and IL-22 present in the sample bind to the capture antibodies on the coated beads. Unbound molecules are washed away during the subsequent wash steps. Fluor-labeled detection antibodies are added to each well and incubated. These detection antibodies recognize and bind to the IL-23 and IL-22 that have been captured onto the beads, thus completing the sandwich. Elution buffer is added to dissociate the protein sandwich, releasing the fluor-labeled antibodies. The eluted antibodies are transferred to a SMC® 384-well Read Plate. The plate is loaded into the FemtoQuest™ System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of IL-23 and IL-22 present in the sample. The amount of IL-23 and IL-22 in unknown samples is interpolated from a standard curve.

Supplies

The SMC® Human IL-23, IL-22 Immunoassay Kit includes all reagents listed below; these components are lot matched and not intended to be used separately. Additional reagents and supplies are required to run this immunoassay, as listed in the next section, Additional Supplies Required (Not provided).

This kit and all reagents supplied are for research use only.

Reagents Included with the Kit

All items are shipped with a cold pack unless otherwise stated.

Description	Storage Conditions	Packaging Details	Component Number
Assay Buffer	2–8 °C	2 x 20 mL	2P-9948-00
IL-23, IL-22 Capture Coated Beads	2–8 °C	1 x 550 µL	2P-2215-00
Standard Diluent	2–8 °C	2 x 20 mL	02-0225-02
IL-23, IL-22 Detection Antibodies	2–8 °C	1 x 270 µL	2P-1215-00
IL-23 Standard	2–8 °C	1 lyophilized vial	2P-8215-01
IL-22 Standard	2–8 °C	1 lyophilized vial	2P-8215-02
IL-23 Quality Control	2–8 °C	1 lyophilized vial	2P-6215-01
IL-22 Quality Control	2–8 °C	1 lyophilized vial	2P-6215-02
10X Wash Buffer	2–8 °C	3 x 50 mL	02-0001-03
Buffer D	2–8 °C	1 x 6 mL	02-0446-00
Elution Buffer B	2–8 °C	1 x 5 mL	02-0211-02
SMC® 2 Plex Commercial Plate	RT	1 plate	02-2PCP-00

Kit Storage

The SMC® 2-plex Human IL-23, IL-22 High Sensitivity Immunoassay Kit should be stored at 2–8 °C. The SMC® 2-plex Commercial Plate can be stored at room temperature (RT).

Discard standards after one use.

Supplied 10X Wash Buffer does not contain preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup® filter, for storage of up to 1 month at 2–8 °C. If not filter sterilized, all remaining 1X wash buffer should be discarded upon experiment completion.

Proper kit performance can only be guaranteed if the materials are stored properly.

Additional Supplies Required (Not provided)

Catalogue numbers provided may be purchased from SigmaAldrich.com or through sales quote, unless otherwise noted.

Equipment

- FemtoQuest™ System for sample acquisition (95-0200-00)
- Orbital microplate shaker for assay plate incubation (for example, Boekel Scientific Jitterbug™ Shaker)
- BioTek® 405™ TSUV Plate Washer for SMC®/MILLIPLEX® Technology (95-0004-06)
- Sphere Mag Plate for performing microparticle capture (90-0003-02)
- Rotisserie tube rotator for microparticle suspension
- Benchtop centrifuge with bucket rotors capable of reaching 1,100 x *g* for sample/plate centrifugation
- Microcentrifuge capable of reaching 13,000 x *g* for reagent/sample centrifugation
- Single channel manual pipettes to accurately dispense 10-20 µL, 20-250 µL and 100-1000 µL
- 12-channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- Plate roller for complete plate sealing (Fisher Scientific, NC9185793)

Supplies

- Micro-centrifuge tubes for sample preparation and storage
- 1 L Container with cap for Wash Buffer dilution
- Stericup® Quick Release Vacuum Filtration System, 0.22 µm, 1 L; for filter sterilizing 1X Wash Buffer (S2GPU11RE)
- MultiScreen® $_{HTS}$ 96-well Plate, hydrophilic PVDF membrane (MSBVN1210)
- 15 mL conical tube with cap for capture bead and Detection Antibody dilution
- Assay Plate: 96-well V-bottom plate for assay setup (AXYP96450VCS)
- Axygen™ Microplate Sealing Film and Tapes (Fisher Scientific, 14-222-344)
- Universal plate cover to minimize plate well contamination (Fisher Scientific, 253623)
- 12-Channel reagent reservoir (sterile) for standard serial dilution (Argos/Cole Parmer, 04395-33)
- VistaLab® 25 mL Reservoirs for addition of reagents (Fisher Scientific, 21-381-27C)
- Millex® Syringe Filter, 0.2 µm for Detection Antibody filtration (SLGPR33RS)
- Luer-Lok® Syringe, 5 mL; for Detection Antibody filtration (Fisher Scientific, 14-829-45)
- Nunc™ Aluminum adhesive plate seals (Fisher Scientific, 276014)

Reagents

- 10X Wash Buffer for automated assay plate washing, 1 L (02-0111-00)
- De-ionized or distilled water for dilution of 10X Wash Buffer

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Assay Best Practices

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. In addition, proper training as well as instrument maintenance is critical for obtaining optimal results in performing SMC® assays. The following notes should be reviewed and understood before the assay is set up.

- Wipe down bench and pipettes with 70% isopropanol before use.
- It is important to allow all reagents to warm to room temperature (RT), 20–25 °C, for approximately 30 minutes.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- The standards prepared by serial dilution must be used within 10 minutes of preparation.

  Note: It is recommended that the standards are prepared as the last step prior to plate setup.
- All washing must be performed with the Wash Buffer provided.
- An orbital microplate shaker for assay plate incubation (for example Boekel Scientific Jitterbug™ Shaker settings #3-5) provide maximal orbital mixing without splashing liquid or causing cross-contamination.
    - Jitterbug™ Shaker setting #3 ~750 rpm
    - Jitterbug™ Shaker setting #4 ~875 rpm
    - Jitterbug™ Shaker setting #5 ~1000 rpm

  Note: If using different orbital shaker, refer to recommended rpm ranges provided for each incubation step, and adjust speeds as necessary to ensure maximal orbital mixing without splashing liquid or causing cross-contamination.
- As the SMC® assay is extremely sensitive to dust particles, do not perform the assay or plate washing under direct airflow.
- Assay Plate must also be protected from light after adding detection antibodies.
- After the assay is complete, seal the SMC® Read Plate before reading immediately or storing temporarily at 2–8 °C. The FemtoQuest™ System requires the use of aluminum adhesive plate seal.
- It is not recommended to store eluted products from SMC® assays overnight at 4 °C or frozen at –80 °C for later reading as performance cannot be guaranteed.
- If SMC® Read Plate has been stored at 4 °C, plate should be left at RT for 30 minutes to 1 hour on the benchtop before reading to avoid a rapid increase in temperature within SMC® Read Plate wells. Bring to RT then centrifuge the plate at 1,100 x *g* for 1 minute prior to reading.
- For optimal FemtoQuest™ System performance, perform Self-Test daily and SMC® Fluorescence Verification Kit monthly.

Precautions

Use caution when handling biological samples. Wear protective clothing and gloves. Components of this reagent kit contain Sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing Sodium azide should be disposed of properly.

Hazard Labels

Ingredient	Catalogue Number	Label	
IL-23 Standard IL-22 Standard IL-23 Quality Control IL-22 Quality Control	2P-8215-01 2P-8215-02 2P-6215-01 2P-6215-02		**Warning.** Harmful if swallowed, in contact with skin or if inhaled. May cause damage to organs (Brain) through prolonged or repeated exposure if swallowed. May cause damage to organs (Respiratory Tract) through prolonged or repeated exposure if inhaled. Harmful to aquatic life with long lasting effects. Do not breathe dust. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. Get medical advice/ attention if you feel unwell. Take off contaminated clothing and wash it before reuse. Dispose of contents/ container to an approved waste disposal plant.
IL-23, IL-22 Capture Coated Beads	2P-2215-00	No Label Required	Harmful to aquatic life. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant.
Assay Buffer	2P-9948-00	No Label Required	Toxic to aquatic life. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Catalogue Number	Label	
Standard Diluent	02-0225-02		**Warning.** May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Do not breathe dust/fume/gas/ mist/ vapours/spray. Get medical advice/ attention if you feel unwell. Dispose of contents/ container to an approved waste disposal plant.
10X Wash Buffer	02-0001-03		**Warning.** Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Elution Buffer B	02-0211-02	No Label Required	Harmful to aquatic life. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant.

Assay Preparation

Sample Preparation

Prepare Serum or Plasma samples by one of the following methods:

Preferred Method: If using a filter plate with prefilter: Stack the filter plate on top of a 96-well receptacle plate. Place 250 µL of sample into a filter plate well and spin for ≥10 minutes at 1,100 x *g*.

If using a microcentrifuge: Centrifuge samples at >13,000 x *g* for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

Sample Dilution

1. Dilute the clarified Serum or Plasma samples 1:2 using the Standard Diluent (For triplicates, transfer 200 µL of clarified sample to the sample preparation plate and add 200 µL Standard Diluent).

1. Use 100 µL per well of 1:2 diluted Serum or Plasma.

2. If further sample dilution is required, samples can be diluted with the provided Standard Diluent.

Reagent Preparation

1. Warm all reagents to RT prior to use for approximately 30 minutes.

2. Store the Detection Antibody away from light until ready to use.

3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:

   - Pour all 3 bottles of 10X Wash Buffer (containing 50 mL each for 150 mL total) into a container capable of holding at least 2 L. Add 1.35 L of deionized water.
   - Mix thoroughly by gentle inversion or with a clean, sterile stir bar.

4. Mix IL-23, IL-22 Antibody Coated Beads on a rotisserie spin rotator, or manually by repeat inversion, for ≥20 minutes until all beads are resuspended.

QC Preparation

1. Gently tap down the bottle to ensure lyophilized product is at the bottom of the bottle. Reconstitute each lyophilized QC in 250 µL of deionized water. Invert the vials several times to mix. Gently pulse vortex the vials for 10 seconds. Allow the vials to sit for 5–10 minutes. Quality Controls are lot matched to the standards and must be used within the same kit lot.

   Note: Quality controls are provided for internal assay performance tracking and are not used for pass/fail criteria of the kit.

2. To prepare the High QC, dilute the IL-23 QC and the IL-22 QC following the instructions in the Certificate of Analysis using Standard Diluent.

3. To prepare the Low QC, dilute 20 µL of the High QC with 480 µL of Standard Diluent.

   Note: If a Mid QC is desired, dilute 100 µL of the High QC with 400 µL of Standard Diluent.

Initial Standard Stock Preparation

1. Gently tap down the bottle to ensure lyophilized product is at the bottom of the bottle. Reconstitute each lyophilized standard in 250 µL of deionized water. Invert the vial several times to mix. Gently pulse vortex the vial for 10 seconds. Allow the vial to sit for 5–10 minutes.

2. Refer to the standard value assignment on the Certificate of Analysis for the Stock concentration of the IL-23 Standard and IL-22 Standard in the vial.

3. Perform the necessary dilutions in Standard Diluent to achieve the final working concentration of 100 pg/mL for IL-23 and 200 pg/mL for IL-22 in a 1.0 mL final volume standard cocktail.

$$\text{Volume IL-23 Stock to Add } (\mu L) = \frac{1{,}000\ \mu L \times 100\ pg/mL}{\text{Stock Concentration } ng/mL} \div \frac{1{,}000\ pg}{ng}$$

$$\text{Volume IL-22 Stock to Add } (\mu L) = \frac{1{,}000\ \mu L \times 200\ pg/mL}{\text{Stock Concentration } ng/mL} \div \frac{1{,}000\ pg}{ng}$$

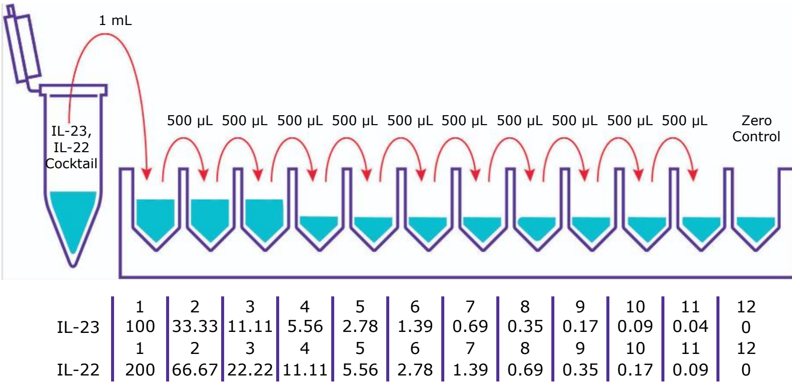
Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform 1:3 serial dilutions of the Standard 1 for Standards 2 through 3 then 1:2 serial dilutions for standards 4 through 11. Standard 12 is the Blank (Standard Diluent only).

Run the standards in triplicate.

Note: Pipette gently into reservoir wells to avoid creating bubbles.

1. Add 1 mL Standard Diluent to wells 2 through 3 of a 12-channel reservoir dilution plate.
2. Add 500 µL Standard Diluent to wells 4 through 12 of a 12-channel reservoir dilution plate.
3. Transfer 1 mL IL-23 and IL-22 working Standard Cocktail (100 pg/mL IL-23 and 200 pg/mL IL-22) (Standard 1) into well 1.
4. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2 stopping at well 11, mixing thoroughly each time. Use a fresh tip with each transfer.



	1	2	3	4	5	6	7	8	9	10	11	12
IL-23	100	33.33	11.11	5.56	2.78	1.39	0.69	0.35	0.17	0.09	0.04	0
	1	2	3	4	5	6	7	8	9	10	11	12
IL-22	200	66.67	22.22	11.11	5.56	2.78	1.39	0.69	0.35	0.17	0.09	0

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Assay Procedure

Target Capture

5. Pipette 100 µL per well of Standards, Quality Controls, or 1:2 diluted Samples to assay plate.
6. Retrieve the IL-23, IL-22 Coated Bead vial from the rotator and transfer its full contents to 11.0 mL of supplied Assay Buffer. Rinse the bead vial with 0.55 mL of fresh Assay Buffer and ensure that all beads have been transferred from the original vial. Mix by gentle inversion. There should be a total volume of 12.1 mL of diluted IL-23, IL-22 Coated Beads.
7. Using a multichannel pipette, add 100 µL per well of diluted IL-23, IL-22 Coated Beads into the assay plate.
8. Seal the assay plate with clear adhesive plate seal, applying pressure to the seal to prevent leaking and cross-contamination.
9. Incubate for 2 hours at 25 °C on microplate incubator/shaker (Jitterbug™ setting #3).
10. Approximately 10 minutes prior to the end of target capture incubation, prepare the IL-23, IL-22 Detection Antibody working stock:
    - **Preferred:** Prepare 1X Detection Antibody by adding 250 µL of 20X Detection Antibody into 4,750 µL of Assay Buffer and filter the diluted Detection Antibody using the syringe with a 0.2 µm filter into a clean tube or clean reagent reservoir.
    - Centrifuge 20X Detection Antibody at 14,000 x *g* for 5 minutes. Prepare 1X Detection Antibody by adding 250 µL of the centrifuged supernatant into 4,750 µL of Assay Buffer.
11. When incubation is complete, centrifuge the assay plate at 1,100 x *g* for 1 minute, place the plate on the washer magnet, and carefully remove clear adhesive plate seal to avoid splashing.

Post-Capture Wash

Wash plate once with a plate washer (BioTek® 405 TSUVS; Post Capture Wash (POSTCAP)). If using automation, please contact your technical service representative for the appropriate automation procedure.

Detection Antibody Incubation

1. After removal from the plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners for 2 minutes.
2. Using a multichannel pipette, dispense 20 µL per well of 1X Detection Antibody without disturbing the bead pellet (It is recommended to change tips).
3. Seal the assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
4. Incubate for 1 hour at 25 °C on microplate incubator/shaker (Jitterbug™ setting #5). Ensure plate is protected from light during this incubation.
5. When incubation is complete, centrifuge at 1,100 *x g* for 1 minute then carefully remove the clear adhesive plate seal to avoid splashing.

Post-Detection Wash

Wash the assay plate 4 times with wash buffer using the 4 cycle Pre-Transfer (4CYCPRE) program on the BioTek® 405 TSUVS washer. If using automation, please contact your technical service representative for the appropriate automation procedure.

Post-Detection Shake

1. After 4 cycle Pre-Transfer wash, visually verify that each well contains ~200 µL of wash buffer.
2. Seal the assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
3. Place the plate on the microplate/incubator shaker (Jitterbug™ setting #3) for 2 minutes. Ensure plate is protected from light during this incubation.
4. Remove the plate from the shaker, and centrifuge at 1,100 *x g* for 1 minute. Carefully remove clear adhesive plate seal to avoid splashing and place it on the plate washer to perform Final Aspiration.

Final Aspiration

Perform Final Aspiration using BioTek® 405 TSUVS; Final Aspirate (FINASP). If using automation, please contact your technical service representative for the appropriate automation procedure.

Elution

1. After removal from the plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners for 2 minutes.
2. Dispense 10 µL Elution Buffer B per well using reverse pipetting without disturbing the bead pellet.
3. Seal assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
4. Incubate the plate for 10 minutes at 25 °C on microplate incubator/shaker (Jitterbug™ setting #5). Ensure plate is protected from light during this incubation.
5. When incubation is complete, centrifuge at 1,100 x *g* for 1 minute.

Assay Reading

To read on the FemtoQuest™ System

1. Place the assay plate with Elution Buffer B onto the sphere mag plate and allow beads to form a tight pellet for 2 minutes.
2. Keeping the assay plate on the magnet, carefully remove the adhesive plate seal. Using a multichannel pipette, add 10 µL of Buffer D to center of wells containing Elution Buffer B. Use a fresh tip with each dispense.
3. Set a manual 12-channel pipette to 18 µL and put 12 tips onto the pipettor. Transfer 18 µL of neutralized eluate solution per well to corresponding wells of the SMC® Read Plate placed over the included plate holder, by aspirating directly from the v-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal the SMC® Read Plate with new clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 x *g*. Remove the seal, inspect reading plate wells and remove bubbles if they are present.
5. Firmly seal the SMC® Read Plate with aluminum plate seal using the recommend plate roller.
6. Remove the plate holder from the sealed SMC® Read Plate and load SMC® Read Plate onto the FemtoQuest™ System. Start read.

   Note:

   - The FemtoQuest™ System will wait (up to 30 minutes) to allow reading plate to equilibrate to internal instrument temperature. The 'Status' message 'Waiting' will be displayed. Once the instrument is ready to read plate, Status will change from 'Waiting' to 'Moving to Well,' to 'Well Scanning'.
   - For assay reading, Analyte 1 is IL-23 and Analyte 2 is IL-22.

SMC® Assay Overview

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100 µL of Standard/Quality Controls/1:2 diluted Serum or Plasma sample and 100 µL of Coated Beads to assay plate.
3. Seal and incubate for 2 hours at 25 °C on appropriate microplate incubator/shaker.

2 hours at 25 °C

4. After capture incubation, centrifuge assay plate at 1,100 x *g* for 1 minute.
5. Perform Post-Capture Wash.
6. Remove from washer magnet and add 20 µL of Detection Antibody per well.
7. Seal assay plate and incubate for 1 hour at 25 °C on microplate incubator/shaker.

1 hour at 25 °C

8. Perform Post-Detection Wash.
9. Seal the assay plate and perform the post-detection shake for 2 minutes on microplate incubator/shaker.
10. Perform the Final Aspiration.
11. Remove from washer magnet and add 10 µL of Elution Buffer B to each well.
12. Seal assay plate and incubate for 10 minutes at 25°C on microplate incubator/shaker.

10 minutes at 25 °C

13. Add 10 µL of Buffer D to neutralize the eluted antibody.
14. Transfer 18 µL of neutralized eluate to the SMC® Read Plate.
15. Seal SMC® Read Plate with aluminum adhesive plate seal for FemtoQuest™ System.
16. Load the FemtoQuest™ System.

Assay Characteristics

Sensitivity

Assay sensitivity measures the true limit of quantitation of an analyte and is often defined by the Lower Limit of Quantification (LLOQ). LLOQ is calculated as the lowest concentration that can achieve CVs of < 20% and the percent recovery of the standard point is still between 80%-120%. The LLOQ of IL-23 is 0.04 pg/mL and the LLOQ of IL-22 is 0.09 pg/mL. Please note that the published LLOQ is data generated during kit verification and can have minor variation between kit lots. For lot specific LLOQ, please see the certificate of analysis.

Refer to Certificate of Analysis for the final QC expected concentrations for both IL-23 and IL-22.

Precision

The assay variations of SMC® IL-23, IL-22 Immunoassay kits were studied using five healthy plasma samples run in triplicate by 3 different operators on 3 different days.

- Mean intra-assay variation was <10% for both IL-23 and IL-22.
- Mean inter-assay variation was <10% for both IL-23 and IL-22.

Cross-Reactivity

This assay was tested for cross-reactivity between IL_23 and IL-22. This assay was tested for cross-reactivity with IL-12p40, IL-12p70, IL-27, IL-35, IL-39, IL-22BP, IL-10, IL-28A (IFN-λ2), IL-28B (IFN- λ3) and IL-29 (IFN- λ1) in addition. IL-23 had less than 5% cross-reactivity to IL-12p40. There was no other cross-reactivity detected with either analyte.

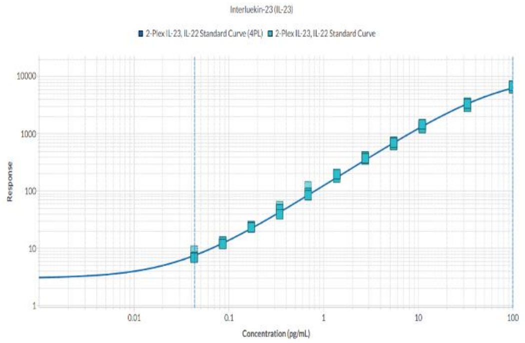
Spike Recovery

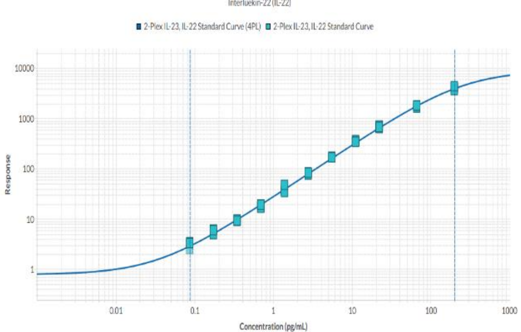
The data represent mean percent recovery of three different concentrations of standard spiked into samples (n = 5 serum samples, 5 plasma samples).

	IL-23		IL-22	
Sample ID	**Serum Recovery**	**Plasma Recovery**	**Serum Recovery**	**Plasma Recovery**
Sample 1	102%	101%	91%	108%
Sample 2	108%	104%	82%	108%
Sample 3	107%	88%	89%	109%
Sample 4	101%	110%	86%	107%
Sample 5	107%	113%	86%	101%
Average	105%	103%	87%	107%

Graph of Typical Reference Curve

Typical FemtoQuest™ Human IL-23 and IL-22 Immunoassay Standard Curve, not to be used to calculate data.





Troubleshooting

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using seal appropriately. Pipette with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected.
		Ensure reagents (including wash buffer) are not contaminated.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	Plate was over-incubated	Confirm plate incubation times are as recommended, particularly for the Detection incubation.
Sample variability is high	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have <2 µL or residual remaining in the well.
	Samples may have high particulate matter or other interfering substances	Samples should be filtered according to the Assay Preparation section. Unprocessed samples could lead to higher imprecision.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing (See Jitterbug™ Shaker setting in Assay Best Practices section).
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x *g* for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed.
		Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

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Problem	Probable Cause	Solution
Beads are lost during the wash	Plate washer needs optimization/cleaning	Contact Tech Support or local Specialist to schedule washer programming. Refer to user guide for cleaning procedure.
	Insufficiently primed washer	Washer should be primed with Wash Buffer prior to running the post capture wash protocol.
	Beads came in contact with water	Washer should be primed with Wash Buffer sufficiently prior to plate wash. Viscosity of water changes the performance of the magnetic particles.
	Proper magnet was not used	Ensure that the SMC® magnetic plate shipped with the BioTek® 405 TSUVS Plate Washer was present on plate wash stage prior to running wash protocol.
Published LLoQ was not achieved	Pipets may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have <2 µL or residual remaining in the well.
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x *g* for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed.
		Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
	Improper dilution/reconstitution of the standard reference material	Confirm appropriate kit protocol was followed when preparing standard curve.
		Ensure standards are prepared less than 10 minutes before starting capture incubation.
		Ensure each serial dilution is thoroughly mixed by pipetting up and down gently several times.

Problem	Probable Cause	Solution
Microparticles do not resuspend into homogenous solution	Beads were not properly stored and may have been frozen	Labelled microparticles should be stored at 4 °C. If microparticles are frozen, they will not resuspend properly.
	Samples may be causing interference due to excess particulate matter	Samples should be properly processed prior to testing to remove particulate matter or lipids.

Well Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
B	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
C	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Standard 11	Standard 12
D	High QC	High QC	High QC	Mid QC	Mid QC	Mid QC	Low QC	Low QC	Low QC	Sample 1	Sample 1	Sample 1
E	Sample 2	Sample 2	Sample 2	Sample 3	Sample 3	Sample 3	Etc.	Etc.	Etc.			
F												
G												
H												

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