Version 1.0

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BT-plex™ Human Cytokine Panel 2

(96-Well Plate Assay**)**

User Manual

Catalog No. X202104A

The BT-plex™ Human Cytokine Panel 2 (12-plex) is a bead-based multiplex assay panel, using magnetic fluorescence-encoded beads on regular flow cytometers. The panel allows simultaneous quantification of Granzyme A, Granzyme B, IFN-ß, IL-9, IL-13, IL-18, IL-22, IL-23, IL-27, MCP-1(CCL2), Soluble CD25 (sCD25 or sIL-2R), and free active TGF-ß1, or any combination of these key cytokines, which, along with targets in BT-plex™ Human Cytokine Panel 1, are involved in major immune responses. The assay provides high sensitivity detection, ease of use, and more robustness in analytical performance compared with similar multiplex assays on the market. This panel has been tested for use on cell culture supernatant, serum, plasma, and other body fluid samples.

The BT-plex™ Human Cytokine Panel 2 is designed to allow flexible customization. Users can select any combination of the targets within the panel. Please visit www.biotimesinc.com for more product information or contact us to discuss your specific needs.

For Research or Further Manufacturing Use Only

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Warning:

1. This reagent may contain Sodium Azide and other common laboratory chemicals. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion.
2. Biological samples should be handled as potentially hazardous. Follow universal precautions as established by the Center for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
3. Do not mix or substitute reagents from different kits or lots. Reagents from different manufacturers should never be used with this kit.
4. Do not use this kit beyond its expiration date.
5. SA-PE and fluorescent beads are light-sensitive. Minimize light exposure.

It is important that you read this entire manual carefully before starting your experiment.

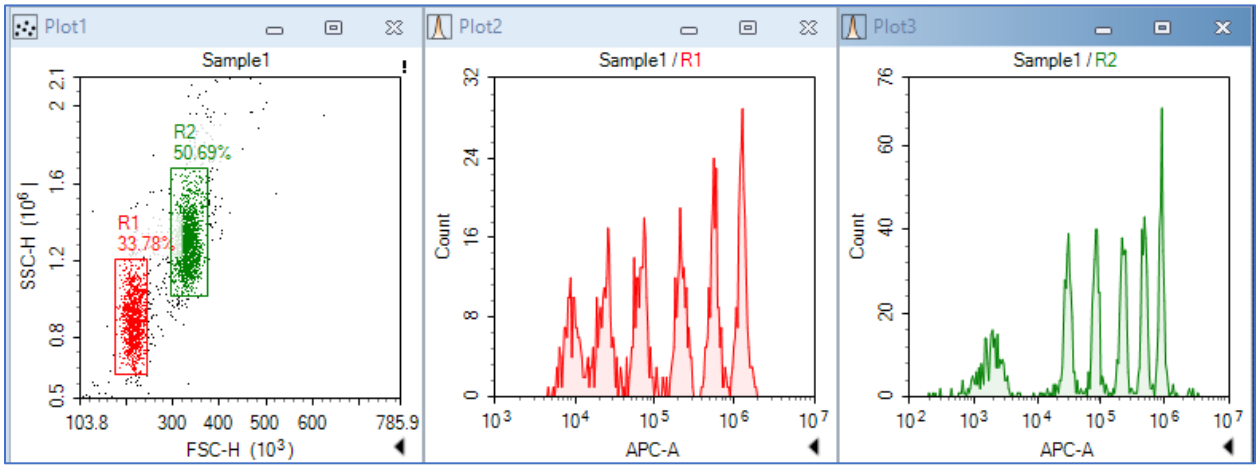
1. ASSAY PRINCIPLES

BT-plex™ bead-based kits use the same principle as typical sandwich immunoassays. Magnetic beads are differentiated by a combination of bead size and internal fluorescence intensities. Each bead set has a functional surface and is chemically conjugated with a specific capture molecule (e.g., antibody or binding protein) on the surface of the bead specific for a given analyte. When a selected group of beads with different Bead IDs are mixed and incubated with a sample, each target analyte in the sample will bind to its specific capture beads. A mixture of biotinylated detection antibodies specific to the targets are added, each detection antibody in the cocktail will bind to its specific analyte bound on the capture beads, thus forming capture bead-analyte-detection antibody sandwiches. Streptavidin-phycoerythrin (SA-PE) subsequently binds to the biotin group on the detection antibodies, providing a fluorescent signal with intensities in proportion to the quantity of bound analytes. With a flow cytometer reader, analyte-specific populations can be segregated and phycoerythrin fluorescent signal can be quantified. The concentration of a particular analyte is determined using a standard curve generated in the same assay.

2. BEADS

The BT-plex assays use two sizes of beads. Each size has several sub-populations that can be identified based on their forward scatter (FSC) and side scatter (SSC) profiles (Beads A, smaller beads and Beads B, larger beads). Each bead size can be further resolved based on their internal fluorescence intensities, which can be detected using FL4, or APC channel, depending on the type of flow cytometer used. The smaller Bead size A consists of 7 bead populations and the larger Bead size B consists of 6 bead populations. Using a total of 13 bead populations distinguished by size and internal fluorescent dye, BT-plex multiplex assays enable simultaneous detection of up to 13 targets in a single sample. A 12-plex panel and associated bead populations are described in Figure 1.

Figure 1. Beads Differentiated by Size and Fluorescence Intensities*



**Gate R1 represent smaller beads (Size A) and Gate R2 represents larger beads (Size B). The R1 bead populations from lower to higher APC intensity are identified as A1, A2, A3, A4, A5, and A6, respectively. The R2 bead populations from lower to higher APC intensity are identified as B1, B2, B3, B4, B5, and B6, respectively.*

For specific conjugated beads used in the Human Cytokine Panel 2, refer to Table 1 for exact assignment of Bead Identifications.

Table 1. Panel Targets, Bead ID, and Reference Standard Concentration

Target	Bead ID	Top Standard Conc.*
Human Granzyme A	A0	5.0 ng/mL
Human Granzyme B	A1	5.0 ng/mL
Human IFN-ß	A3	5.0 ng/mL
Human IL-9	A4	5.0 ng/mL
Human IL-13	A5	5.0 ng/mL
Human IL-18	A6	5.0 ng/mL
Human IL-22	B1	5.0 ng/mL
Human IL-23	B2	5.0 ng/mL
Human IL-27	B3	5.0 ng/mL
Human MCP-1	B4	5.0 ng/mL
Human sCD25	B5	5.0 ng/mL
Human TGF-ß1	B6	5.0 ng/mL

Bead ID is used to associate bead populations with target analytes. For data analysis of FCS files, association of analyte and bead ID are defined during the gating step of the data analysis.

**The concentrations of the target cytokines when the lyophilized standard is reconstituted to 0.5 mL in Assay Buffer.*

3. STORAGE

All original kit components should be stored at 2-8°C. DO NOT FREEZE Pre-mixed Beads, Detection Antibodies or SA-PE.

Once the standard and Control Samples have been reconstituted, transfer contents into polypropylene microfuge vials. DO NOT STORE RECONSTITUTED STANDARD and Control Samples IN GLASS VIALS. Leftover reconstituted standard and Control Samples should be stored at ≤-70°C for future use. Avoid multiple freeze-thaw cycles. Discard any leftover diluted standards.

4. MATERIALS SUPPLIED

The BT-plex kit contains enough reagents for 100 tests with a small overfill to cover pipetting loss. When assays are performed in duplicate, reagent volume provided is enough for assays with an 8-point standard curve and 40 samples.

Table 2: Kit Components information

Kit Components	Quantity	Volume	Part No.
Human Cytokine Panel 2 Pre-mixed Beads, Magnetic	1 Bottle	3.5 mL	X202104A-01 or vary*
Human Cytokine Panel 2 Detection Antibody	1 Bottle	3.5 mL	X202104A-02
Human Cytokine Panel 2 Standard, Lyophilized	1 Bottle	NA	X202104A-03
BT-plex™ Streptavidin-Phycoerythrin	1 Bottle	3.5 mL	X202104A-04
BT-plex™ Assay Buffer	1 Bottle	25 mL	BTAB01
BT-plex™ 20X Wash Buffer	1 Bottle	25 mL	BTWB01
Human Cytokine Panel 2 Control Sample 1, Lyophilized***	1 Bottle	NA	X202104A-05
Human Cytokine Panel 2 Control Sample 2, Lyophilized***	1 Bottle	NA	X202104A-06
Low binding V-bottom plate**	1 Plate	NA	NA
Adhesive Plate sealer	2 sheets	NA	NA
Instruction Manual	1	NA	NA

*Catalog number of premixed beads vary with selection of targets. X202104A-01 contains all 12 targets. Premixed beads are ready to use.

**Low-binding V-bottom plate can be reused once after thorough cleaning.

***Human Cytokine Panel 2 Control Samples are available separately for use as quality controls for the assays. Not all kits are provided with Control Samples.

Table 3: Customization of Targets

Target	Bead ID*	Target to select
Human Granzyme A	A0	
Human Granzyme B	A1	
Human IFN-ß	A3	
Human IL-9	A4	
Human IL-13	A5	

Human IL-18	A6	
Human IL-22	B1	
Human IL-23	B2	
Human IL-27	B3	
Human MCP-1	B4	
Human sCD25	B5	
Human TGF-ß1	B6	

5. MATERIALS TO BE PROVIDED BY THE USERS

5.1. A flow cytometer with two lasers (e.g., a 488 nm blue laser or 532 nm green laser and a 633-635 nm red laser) capable of distinguishing 575 nm and 660 nm Examples of compatible flow cytometers are listed below:

Flow Cytometer*	Classification Channel	Emission Wavelength	Reporter Channel	Emission Wavelength
Agilent Novocyte™	APC	660 nm	PE	572 nm
Beckman Coulter-CytoFLEX™	APC	660 nm	PE	585 nm
Beckman Gallios™	APC	660 nm	PE	575 nm
BD Accuri™ C6/ C6 Plus	APC, FL4	675 nm	PE, FL2	585 nm
BD FACSCalibur™, Canto™, Canto II, LSR™, LSRII™, LSR Fortessa™, FACSAria™	APC/FL4	660 nm	PE/FL2	575 nm
Cytek Aurora™ with YG Laser	R4	720 nm	PE, YG1	575 nm

**For setting up various flow cytometers, please refer to the instrument instructions provided by manufacturer.*

5.2. Multichannel pipettes capable of dispensing 25 µL to 200 µL
5.3. Reagent reservoirs for multichannel pipetting
5.4. Polypropylene microfuge tubes (1.5 mL), mini-FACS tubes
5.5. Vortex mixer
5.6. Aluminum foil
5.7. Absorbent pads or paper towels
5.8. Orbital plate shaker (e.g., Lab-Line Instruments, model #4625)
5.9. Table-top centrifuges (e.g., Eppendorf centrifuge 5415) for microfuge tubes
5.10. Magnetic separator for V Bottom Plate (e.g., Invitrogen A14179)
5.11. If filter plate is used, a vacuum filtration unit (e.g., Millipore MultiScreen ® HTS Vacuum Manifold, cat # MSVMHTS00 or equivalent) and a vacuum source.

5.12. If a magnetic 96-well plate separator is not available, a centrifuge with a swinging bucket adaptor for microtiter plates (e.g., Beckman Coulter Allegra™ 6R Centrifuge with MICROPLUS CARRIER adaptor for GH3.8 and JS4.3 Rotors)

6. ASSAY PREPARATION

6.1. Preparation of Serum Samples:

6.1.1. Allow the blood to clot for 30 minutes or longer and centrifuge for 10 minutes at 1,000 x g.

6.1.2. Remove serum and assay immediately or aliquot and store samples at ≤-20°C. Avoid multiple freeze/thaw cycles.

6.1.3. When using frozen samples, it is recommended that samples are thawed completely, mixed, and centrifuged to remove particulates prior to use.

6.2. Preparation of Plasma Samples:

6.2.1. Plasma collection using EDTA or citrate as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1,000 x g within 30 minutes after blood collection.

6.2.2. Remove plasma and assay immediately, or aliquot and store samples at ≤-20°C. Avoid multiple freeze/thaw cycles.

6.2.3. When using frozen samples, it is recommended that samples are thawed completely, mixed well, and centrifuged to remove particulates.

6.3. Preparation of Tissue Culture Supernatant:

Centrifuge the sample to remove debris and assay immediately. If not possible, aliquot and store samples at ≤-20°C. Avoid multiple (>2) freeze/thaw cycles.

6.4. Preparation of Antibody-Immobilized Beads

6.4.1. If pre-mixed beads are provided in the kit, vortex the bead bottle for 30 seconds prior to use.

6.4.2. If individual beads are provided in the kit, the individual beads should be mixed with each other and diluted to 1X final concentration with Assay Buffer prior to use. To mix the diluted beads, follow the instructions below (a 7-plex custom panel is used as an example)

6.4.2.1. Vortex each bead vial for 30 seconds to completely resuspend the beads.

6.4.2.2. Calculate the amount of mixed and diluted beads needed for the assay. Prepare extra to compensate for pipetting loss.

6.4.2.3. Each reaction needs 25 µL of mixed and diluted beads. For 50 reactions, 1.5 mL of mixed beads is needed (1.25 mL plus 0.25 mL extra volume). For 96 reactions, prepare 3.0 mL of mixed beads.

6.4.2.4. As an example, to make 3.0 ml of 7-plex 1X diluted beads, transfer 0.25 mL of each of the 7 individual beads (12X concentrated) to a fresh tube (total bead volume = 1.75 mL) and add 1.25 mL of Assay Buffer to make the final volume of 3.0 mL.

6.5. Preparation of Wash Buffer

6.5.1. Bring the 20X Wash Buffer to room temperature and mix to bring all salts into solution.

6.5.2. Dilute 25 mL of 20X Wash Buffer with 475 mL deionized water. Store unused portions between 2°C and 8°C for up to one month.

6.6. Standard Preparation

6.6.1. Prior to use, reconstitute the lyophilized Human Cytokine Panel 2 Standard with **0.5 mL Assay Buffer**.

6.6.2. Mix well and allow the vial to sit at room temperature for 10 minutes.
6.6.3. Transfer the reconstituted standard to an appropriately labeled polypropylene microfuge tube. This will be used as the top standard S7. Note: The top standard concentrations of analytes in this kit are set at the same concentrations. But the top concentrations for each target may subject to change from lot to lot (see lot-specific information for details).
6.6.4. Label 6 polypropylene microfuge tubes as S6, S5, S4, S3, S2 and S1, respectively.
6.6.5. Add 300 µL of Assay Buffer to each of the six tubes.
6.6.6. Prepare 1:4 dilution of the top standard (S7) by transferring 100 µL of the top standard (S7) to the S6 tube and mix well. This will be the S6 standard.
6.6.7. In the same manner, perform serial 1:4 dilutions to obtain S5, S4, S3, S2 and S1 standards (see the table below using the top standard at 5,000 pg/mL as an example).
6.6.8. Assay Buffer will be used as the 0 pg/mL standard (C0).

Standard	Serial Dilution	Assay Buffer to Add	Standard To Add	Standard Conc. (pg/mL)
S7 (Original)	NA	0.5 mL to reconstitute lyophilized standard	NA	5,000.0
S6	1:4	150 µL	50 µL of S7	1,250.0
S5	1:16	150 µL	50 µL of S6	312.5
S4	1:64	150 µL	50 µL of S5	78.1
S3	1:256	150 µL	50 µL of S4	19.5
S2	1:1,024	150 µL	50 µL of S3	4.9
S1	1:4,096	150 µL	50 µL of S2	1.2
S0	NA	150 µL	NA	0

A user may choose to make different serial dilutions (e.g., 5,000, 2,500, 1,250, 625, 312.5, 156.3, 78.2, 39.1, 19.6, 9.8, 4.9, 2.4, and 0 pg/mL) or using a different starting top concentration. In this case, reconstitution volume and dilution factors need to be adjusted accordingly.

6.7. Preparation of Control Samples (Optional)

Human Cytokine Panel 2 Control Sample 1 and Control Sample 2 should be reconstituted to 0.5 mL each with Assay Buffer. The reconstituted Control Samples can be tested as regular samples.

6.8. Sample Dilution

6.8.1. Serum or plasma samples must be diluted 5-fold with Assay Buffer before testing (e.g., dilute 20 µL of sample with 80 µL of Assay Buffer).
6.8.2. For cell culture supernatant samples, the levels of cytokines can vary greatly from sample to sample. A preliminary experiment may be needed to determine the appropriate dilution factor. If sample dilution is desired, dilution should be done with corresponding fresh cell culture medium or Assay Buffer to ensure accurate measurement.

7. ASSAY PROCEDURE

The BT-plex™ assay can be performed in a low-binding V-bottom plate, U-bottom plate, flat bottom plate, a filter plate, or in small test tubes (e.g., microfuge tubes, mini-FACS tubes). Low-

binding plates are typically made of polypropylene materials. Plates made of polystyrene materials such as cell culture plates, or ELISA plates should not be used for bead assays.

A low-binding V-Bottom 96-well plate is provided with each kit. The following assay protocols are described based on V-Bottom plate. For using individual small test tubes to run the assays, the protocols can be easily modified. Instead of using a magnetic plate separator, use centrifugation to separate the beads. For use with a filter plate (not provided) or for further modification of protocols, please contact our technical support team (support@biotimesinc.com) to discuss recommendations.

Performing the Assay Using a V-, U-bottom Plate

- Allow all reagents to warm to room temperature (20-25°C) prior to use.
- Keep the plate upright during the entire procedures (except for quick decanting step) to avoid bead loss.
- The plate should be placed in the dark or wrapped with aluminum foil for all incubation steps.
- Ideally, standards and samples should be run in duplicate and arranged on the plate and read on a flow cytometer in a consistent sequential order so that FCS file names and sample IDs can be easily identified during data analysis.
- If an autosampler is used for plate reading, the reading orientation and sequential order should be carefully programed so that it matches the desired plate map.

7.1. Add **25 µL of diluted standards** to corresponding standard wells of the V-Bottom Plate.

7.2. Add **25 µL of samples** (properly diluted) to sample wells.

7.3. Vortex mixed beads for 30 seconds. Add **25 µL of the Human Cytokine Panel 2 Premixed Beads** to each well. (Note: During beads addition, shake mixed beads bottle intermittently).

7.4. Seal the plate with a sheet of adhesive plate sealer. Cover the entire plate with aluminum foil to protect the plate from light.

7.5. Shake the plate at approximately 500-800 rpm on an orbital plate shaker for 5 min at room temperature. Then place the wrapped plate in a refrigerator **(2-8°C) and incubate overnight (e.g., 16-18 h)**. The overnight incubation can be performed with or without shaking. Depending on type of shaker, the speed should be high enough to keep beads in suspension during incubation steps, but not too high to cause spill-over of liquids from wells.

7.6. Place the plate on a magnetic 96-well plate separator, wait for 2 min. Carefully remove liquid from the wells by decanting while holding the V-bottom plate on top of the magnetic 96-well separator held tightly together, then blotting the plate on paper towels. Remove liquids as much as possible.

Note: If a magnetic 96-well plate separator is not available, centrifuge the plate at 250 x g for 5 minutes with a swinging bucket rotor. Immediately after centrifugation, decant the liquid into a container by quickly inverting and flicking the plate in one continuous and forceful motion. Blot the plate on a stack of clean paper towel and drain the remaining liquid from the well as much as possible.

7.7. Add **25 µL of Human Cytokine Panel 2 Detection Antibody** to each well. Cover the entire plate with aluminum foil to protect the plate from light.

7.8. Shake the plate at approximately 500-800 rpm on an orbital plate shaker for 1 **hours at room temperature**.

7.9. **Do not wash the plate!** Add **25 µL of SA-PE** to each well directly. Seal the plate with the plate sealer. Wrap the entire plate with aluminum foil and shake the plate on a plate shaker for additional **30 minutes** at room temperature.
7.10. Repeat the Washing Step as described in 7.6.
7.11. Add 150 µL of 1X Wash Buffer to each well.
7.12. Read samples on a flow cytometer. If the flow cytometer is equipped with an autosampler, the samples can be read directly from the plate. Please be sure to program the autosampler to resuspend beads in the well immediately before taking each sample. If an autosampler is not available, the samples can be transferred from the plate to micro FACS (or FACS) tubes and read manually on the flow cytometer.

8. INSTRUMENT SETUP

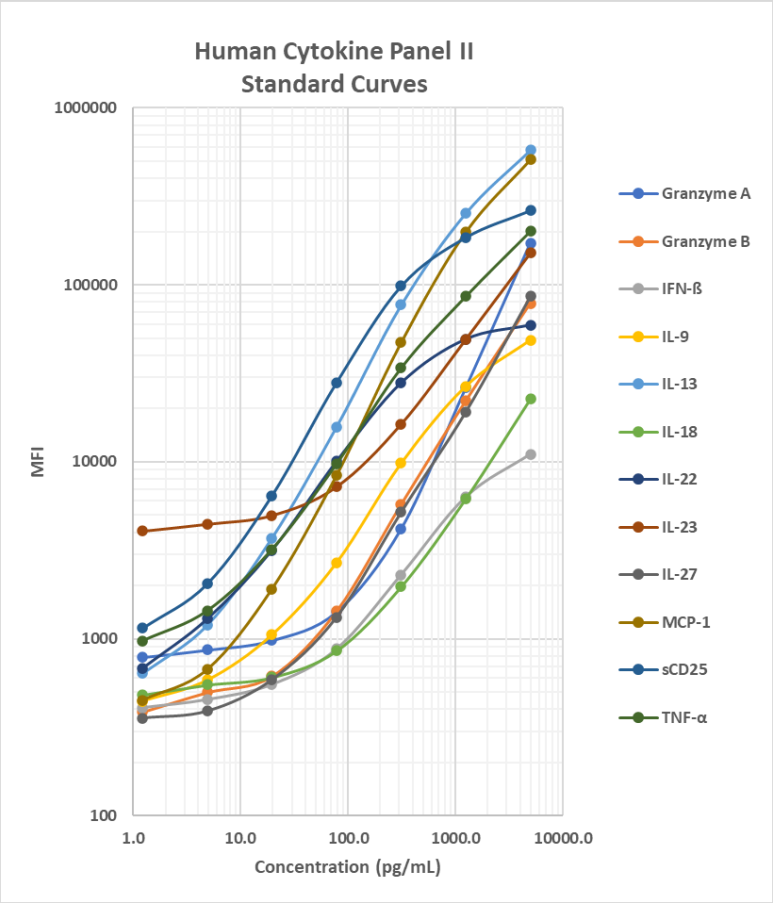
8.1. Start up the instrument following the instrument manufacturer's recommendations.
8.2. Create a template for data acquisition using instrument's data acquisition software. A template is a document or worksheet with density plots (e.g., FSC vs. SSC; PE vs. APC plots) that allows the user to perform machine setup and data acquisition.
8.3. Set up the PMT voltages of each channel, if necessary, to be used for data acquisition using the S0 and S7 wells of standards. Some digital flow cytometers have fixed PMT settings (e.g., Accuri C6™ and Novocyte™) and no PMT adjustment is needed.

9. DATA ACQUISITION AND ANALYSIS

9.1. Before sample reading, make sure that the flow cytometer is set up properly.
9.2. Create a new template or open an existing template (for details on how to create a cytometer-specific template, please refer to the Flow Cytometer Setup Guide).
9.3. Vortex each sample for 5 seconds before analysis for manual reading. Set plate agitation prior to each reading.
9.4. Set the flow rate to low. Set the number of gated beads to be acquired to about 150-200 per analyte (e.g., acquire 2,400 beads for a 12-plex assay). Instead of acquiring data using total events, create a large gate to include both Beads A and Beads B (gate A+B) and set to acquire the number of events in gate A + B. This will exclude majority of the debris from samples.
9.5. Read samples.
9.6. If using an auto-sampler, read column by column (A1, B1, C1...A2, B2, C2.., A3...).
9.7. When naming data files, try to use simple names with a consecutive numbering for easy data traceability and analysis.
9.8. Store all FCS files in the same folder for each assay. If running multiple assays, create a separate folder for each assay.
9.9. Proceed to data analysis using appropriate Data Analysis Software when data acquisition is completed.
9.10. The FCS file generated on a flow cytometer should be analyzed using a compatible data analysis software package for FCS files. Contact support@biotimesinc.com for proper Data Analysis Software.
9.11. FCS files may need to be exported for some flow cytometers. The data can be exported as FCS 3.0, FCS 3.1 format.
9.12. BioTimes, Inc. offers free of charge data analysis for our customers. Customers can zip FCS files and send the data package to support@biotimesinc.com, or put the files in a secure shared location such as Dropbox, along with detailed instructions on standard concentrations, plate map, and sample replicate information. Upon timely communication with us, we will analyze the data and send the results back.

10. ASSAY CHARACTERIZATION

10.1. Representative Standard Curve



This standard curve was generated using the BT-plex Human Cytokine Panel 2 for demonstration purpose only. A standard curve must be run with each quantitative assay.

10.2. Assay Sensitivity

The assay sensitivity (minimum detectable concentration, MinDC) is the theoretical limit of detection calculated using the Data Analysis Software by applying a 5-parameter curve fitting algorithm with weighting. The typical assay sensitivity data below is for reference use only.

Sensitivity	MinDC (pg/mL)
Granzyme A	5.3
Granzyme B	1.6
IFN-ß	1.5
IL-9	0.8
IL-13	1.3
IL-18	1.6
IL-22	0.2
IL-23	3.5
IL-27	1.8
MCP-1	0.5
sCD25	0.5
TNF-α	0.4

10.3. Cross-Reactivity

Twenty-six recombinant human cytokines and over 100 native human serum proteins were tested at ≥ 50 ng/mL using the BT-plex Human Cytokine Panel 2. There is no detectable cross-reacting signal among targets within the panel or with non-targeted proteins.

10.4. Accuracy (Spike Recovery)

Target proteins with known concentrations were spiked into 5 properly diluted serum samples at four different concentrations within the assay range. The spiked samples were then tested. The measured concentrations were compared with the spiked quantity. The overall recovery % of spiked proteins are within acceptable ranges for multiplex assays.

Target	Spike and Recovery, %
Granzyme A	100.9
Granzyme B	123.6
IFN-ß	63.9
IL-9	69.7
IL-13	69.3
IL-18	64.8
IL-22	55.0
IL-23	66.7
IL-27	90.0
MCP-1	78.0
sCD25	85.6
TGF-ß1	75.8

10.5. Intra-Assay Precision

Two independent samples were measured 8 times on the same plate. The overall intra-assay CV% is generally acceptable.

Target	Intra-Assay CV%
Granzyme A	10.4
Granzyme B	16.7
IFN-ß	7.1
IL-9	6.3
IL-13	2.4
IL-18	3.7
IL-22	6.7
IL-23	2.9
IL-27	11.7
MCP-1	5.9
sCD25	4.6
TGF-ß1	9.5

10.6. Inter-Assay Precision

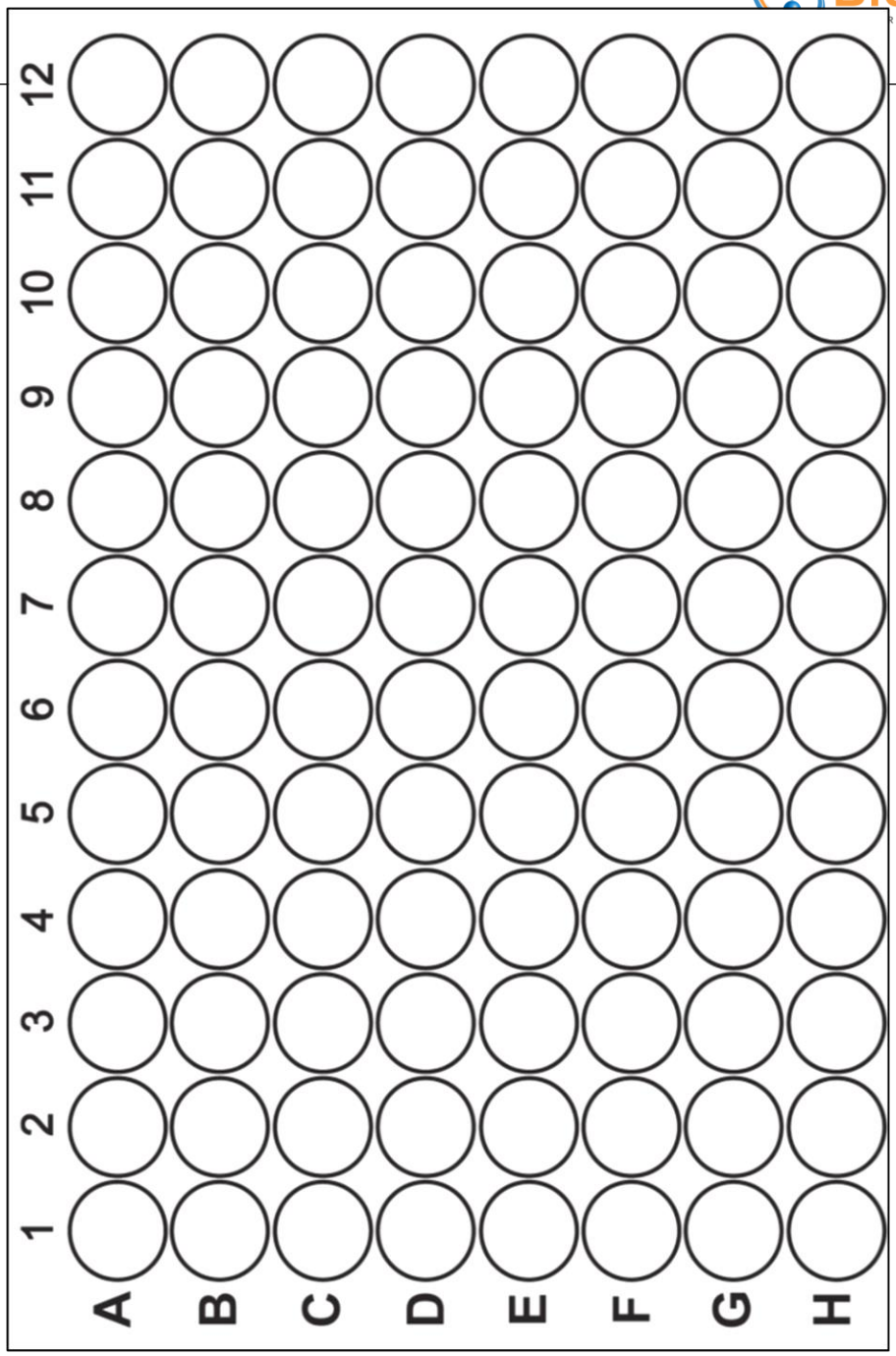
Overall inter-assay CV is within 15% for all targets.

10.7. Biological Samples

Cytokine Concentrations in serum samples from 10 presumptive healthy donors and 10 presumptively recovered CoVID-19 patients were determined and concentrations reported.

Sample Type	Serum Sample	IL-13	IL-9	IL-22	Granzyme A	Granzyme B	sCD25	IFNß	IL-18	MCP-1	TGF-ß1	IL-23
		pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
"Healthy" Subjects	1	1.35	265.52	<1.83	251.06	271.95	118.22	<0.62	561.99	13.42	107.29	104.42
	2	<0.47	5.6	<1.83	10.79	<1.90	11.98	<0.62	410.44	<0.62	<1.04	1.49
	3	<0.47	<0.88	<1.83	1.39	4	18.71	<0.62	258.52	<0.62	<1.04	1.31
	4	<0.47	330.65	<1.83	283.24	12.41	9.18	<0.62	552.74	7.75	6.29	2.38
	5	<0.47	<0.88	<1.83	5.62	<1.90	1.97	<0.62	209.89	0.63	<1.04	3.99
	6	<0.47	<0.88	<1.83	<0.78	<1.90	10.17	<0.62	236.99	<0.62	<1.04	<0.74
	7	<0.47	<0.88	<1.83	3.01	6.31	16.56	<0.62	265.09	<0.62	<1.04	2.91
	8	<0.47	24.65	<1.83	29.65	<1.90	2.54	<0.62	298.02	0.94	<1.04	1
	9	<0.47	<0.88	<1.83	<0.78	<1.90	3.88	<0.62	485.5	<0.62	<1.04	<0.74
	10	0.59	16.86	<1.83	28.05	26.48	34.61	<0.62	218.88	5.18	<1.04	5.55
Recovered Covid-19 Subjects	11	1.98	76.03	<1.83	87.61	181.04	74.57	4.81	838.87	9.62	43.39	60.01
	12	1.6	41.49	<1.83	79.1	84.21	42.79	2.56	5178.26	5.47	7.6	30.75
	13	3.49	134.65	<1.83	143.76	126.21	75.46	1.94	796.28	15.44	28	39.62
	14	2.71	85.15	<1.83	87.53	192.16	72.47	5.74	754.85	10.67	37.2	105.57
	15	<0.47	<0.88	<1.83	47.93	<1.90	7.09	<0.62	308.16	<0.62	14.76	2.79
	16	<0.47	2.49	<1.83	18.45	14.42	2.29	<0.62	243.67	2.58	<1.04	5.76
	17	7.42	386.08	3.44	257.98	242.78	104.79	1.31	915.29	34.08	98.97	67.41
	18	<0.47	1.11	<1.83	9.15	8.23	3.34	<0.62	2874.21	1.04	14.61	3.01
	19	3.21	110.62	<1.83	120.13	195.66	67.65	6.78	649.43	13.32	60.77	90.14
	20	2.29	100.12	<1.83	128.81	260.9	93.16	9.95	7798.95	13.75	75.29	111.39

In addition, the Human Cytokine Panel 2 panel were biologically validated using human PBMC cells cultured with various known conditions for stimulating immune responses (LPS, CD3/CD28, Poly I:C, PHA, PMA, etc.) and the data were consistent with literature reported responses for each target.



BT-plex™ Human Cytokine Panel 2 is manufactured by BioTimes, Inc.,

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