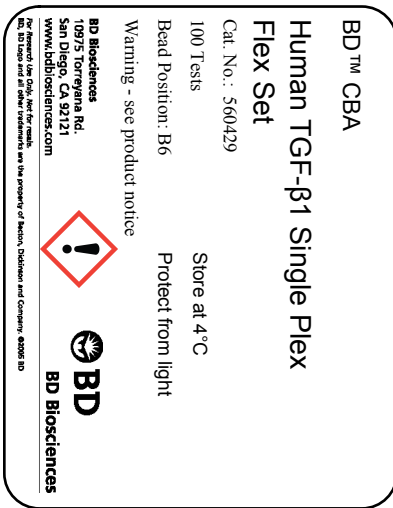


Technical Data Sheet

Human TGF-β1 Single Plex Flex Set



Product Information

Material Number:	560429
Size:	100 Tests
Bead Position:	B6
Assay Range:	40-10,000 pg/mL
Reactivity:	QC Testing: Human Reported Reactivity: Mouse, Rat, Pig, Cow

Component Description: Human TGF-β1 Standard
 Component Mat. No: 51-9006180
 Component Storage Buffer: Lyophilized in an aqueous buffered solution containing BSA and ProClin™ 150.

Component Description: Human TGF-β1 PE Detection Reagent
 Component Mat. No: 51-9006182
 Component Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Component Description: Human TGF-β1 Capture Bead B6
 Component Mat. No: 51-9006184
 Component Storage Buffer: Aqueous buffered solution containing fetal bovine serum and ≤0.09% sodium azide.

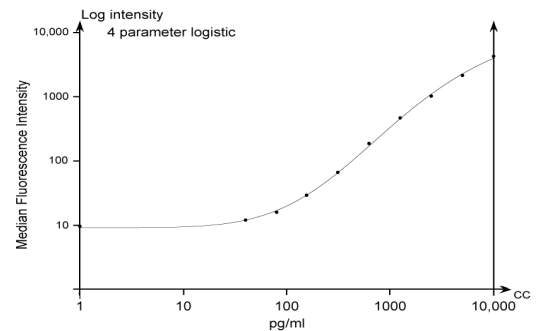


Figure 1. Example BD CBA Human Soluble Human TGF-β1 Flex Set standard curve. Data acquired on a BD FACSAArray bioanalyzer and analyzed using the FCAP Array Software (Cat. No. 641488).

Description

The BD™ Human TGF-β1 Flex Set is a bead-based immunoassay capable of measuring soluble human TGF-β1 in serum and cell culture supernatant samples. Human reactivity was determined by testing samples with the BD CBA Human TGF-β1 Flex Set. The biology and function of TGF-β1 has been extensively reviewed in the literature.

The BD CBA Human TGF-β1 Flex Set is a single plex assay. The BD CBA Human TGF-β1 Flex Set should not be used with the following:

- BD CBA Human, Mouse or Rat Soluble Protein Flex Sets
- BD CBA Cell Signaling Flex Sets
- BD CBA Human Soluble Receptor Protein Flex Sets
- BD CBA Human Immunoglobulin Flex Sets
- Non-BD Bead Based Assays

Application Notes

Recommended Assay Procedure: TGF-β1 Activation Reagent Preparation

The following reagents are needed to activate the latent TGF-β1 to its immunoreactive form:

Reagents for cell culture supernatant samples

1. 1 N HCl (100mL) - To 91.67 mL deionized water, slowly add 8.33 mL of 12 N HCl. Mix well.
2. 1.2 N NaOH/0.5 M HEPES (100mL) - To 75 mL of deionized water, slowly add 12 mL of 10 N NaOH. Mix well. Add 11.9 g of HEPES. Mix well. Bring the final volume to 100 mL with deionized water.

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Reagents for serum and plasma samples

1. 2.5 N Acetic acid/8 M Urea (100mL) - To 40 mL of deionized water, slowly add 48.1 g of Urea. Mix until dissolved. Slowly add 14.4 mL of glacial acetic acid. Mix well. Bring the final volume to 100 mL with deionized water.
2. 2.7 N NaOH/1 M HEPES (100mL) - To 56 mL deionized water, slowly add 27 mL of 10 N NaOH. Mix well. Add 23.8 g of HEPES. Mix well. Bring the final volume to 100 mL with deionized water.

TGF-β1 Sample Activation

To activate the latent TGF-β1 to its immunoreactive form, samples have to be acidified to pH 3.0 or lower for a period of time and neutralized back to pH 7.2 - 7.6 before testing. Use polypropylene tubes. Do not activate the Flex Set standard. Recommended dilutions are a guideline, optimization may be required.

Activation of cell culture supernatant samples

1. Add 0.1 mL of 1 N HCl to 0.5 mL of sample and mix well.
2. Incubate for 10 minutes at RT.
3. Neutralize by adding 0.1 mL of 1.2 N NaOH/0.5 M HEPES and mix well.
4. The sample is now ready for testing.
5. The calculated concentration of samples run in the assay should be multiplied by a dilution factor of 1.4.

Activation of serum samples

1. Add 0.1 mL of 2.5 N Acetic Acid/8 M Urea to 0.1 mL of sample and mix well.
2. Incubate for 10 minutes at RT.
3. Neutralize by adding 0.1 mL of 2.7 N NaOH/1 M HEPES and mix well.
4. Dilute serum samples 10-fold with assay diluent prior to performing the assay.
5. The calculated concentration of samples run in the assay should be multiplied by a dilution factor of 30.

Note: TGF-β1 is highly conserved among species and this assay cross-reacts with multiple species, including mouse, rat, bovine, and porcine. Therefore, conditioned medium containing mammalian serum should be expected (when acidified) to have a significant background level of TGF-β1, which may vary depending on the species of the serum, batch, and percentage of serum used. There are several ways to deal with this background level of TGF-β1:

1. Determine the background TGF-β1 level in the culture medium alone and subtract it from the sample values.
2. Use mammalian serum-free medium for cell culture.
3. Switch to serum-free medium for desired period of time before harvesting samples.

Preparation of BD CBA Human TGF-β1 Flex Set Standard

A standard curve will need to be prepared. The protocol below indicates how the standard should be diluted for use in a BD CBA Human TGF-β1 Flex Set. Please follow the instructions below as they differ from those provided in the Human Soluble Protein Master Buffer Kit Manual. Store lyophilized standard and other components at 4°C.

1. Open one vial of lyophilized standard.
2. Transfer the lyophilized standard sphere into a polypropylene tube (Recommended 15 mL Conical Tube, BD Falcon™ Cat.No.352097). Label tube "Top Standard". **Investigators are strongly advised to transfer the lyophilized standard to the polypropylene tube for reconstitution as other methods may result in increased variability.**
4. Reconstitute the standard with 1.0 mL of Assay Diluent. Allow the reconstituted standard to equilibrate for at least 15 minutes before making dilutions. Mix reconstituted protein by pipette only. Do not vortex or mix vigorously. When reconstituted in 1.0 ml of Assay Diluent, the standard has a protein concentration of 10,000 pg/ml. Discard unused reconstituted standard, do not store or reuse.
5. Label 12 × 75 mm tubes (BD Falcon™ Cat.No.352008) and arrange them in the following order: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256.
6. Pipette 500 µL of Assay Diluent to each of the remaining tubes.
7. Perform a serial dilution by transferring 500 µL from the Top Standard to the 1:2 dilution tube and mix thoroughly (Mix by pipette only, do not vortex). Continue making serial dilutions by transferring 500 µL from the 1:2 tube to the 1:4 tube and so on to the 1:256 tube. Prepare one tube containing Assay Diluent to serve as the 0 pg/mL negative control.
8. It is recommended that the first ten wells or tubes in the experiment be the standards. Standards should be run in order from least concentrated (0 pg/ml) to most concentrated (Top Standard).

Preparation of BD CBA Human TGF-β1 Flex Set Capture Beads

The Capture Beads provided in the BD CBA Human TGF-β1 Flex Set are at a 50× concentration and must be diluted to their optimal concentration in the Capture Bead Diluent buffer before adding to a given assay tube or assay well. **The Capture Bead Diluent for Serum/Plasma provided in the Human Soluble Protein Master Buffer Kit is not used in this assay, even if testing serum or plasma samples.**

1. Determine the number of tests in the experiment. It is recommended that the user prepare a few additional tests than they will use in the experiment to ensure that there is enough material prepared for the experiment.
2. Vortex the Capture Beads stock vial for at least 15 seconds to resuspend the beads thoroughly.

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- Determine the total volume of diluted beads needed for the experiment. Each tube/well requires 50 μL of the diluted beads. The total volume of diluted beads can be calculated by multiplying the number of tests (determined in step 1) by 50 μL .
- Determine the volume needed for the Capture Beads. Beads are supplied so that 1.0 μL = 1 test. Therefore, the required volume (μL) of beads is equal to the number of tests.
- Determine the volume of Capture Bead Diluent needed to dilute the beads. The volume of Capture Bead Diluent can be calculated by subtracting the volume of beads from the total volume of diluted beads needed to perform the assay.
- Pipette the Capture Beads and Capture Bead Diluent into a tube labeled Diluted Capture Beads.

Preparation of BD CBA Human TGF- β 1 Flex Set PE Detection Reagent

The PE Detection Reagent provided with the BD CBA Human TGF- β 1 Flex Set is a 50 \times bulk (1 μL /test) and should be diluted to its optimal volume per test (50 μL /test) before adding the PE Detection Reagent to a given tube or assay well.

Note: Protect the PE Detection Reagent from exposure to direct light because they can become photobleached and will lose fluorescent intensity.

- Determine the number of tests to be run in the experiment. It is recommended that the user prepare a few additional tests than they will use in the experiment to ensure that there is enough material prepared for the experiment.
- Determine the total volume of diluted PE Detection Reagent needed for the experiment. Each tube/well requires 50 μL of the diluted PE Detection Reagent. The total volume of diluted PE can be calculated by multiplying the number of tests (determined above).
- Determine the volume needed for the TGF- β 1 PE Detection Reagent. The PE Detection Reagent is supplied so that 1.0 μL = 1 test. Therefore, the required volume (μL) of PE Detection Reagent is equal to the number of tests.
- Determine the volume of Detection Reagent Diluent needed to dilute the PE Detection Reagents. The volume of Detection Reagent Diluent can be calculated by subtracting the volume of the TGF- β 1 PE Detection Reagent tested from the total volume of diluted PE needed.
- Pipette the Detection Reagent and Detection Reagent Diluent into a tube labeled TGF- β 1 PE Detection Reagent. Store at 4 $^{\circ}\text{C}$, protected from light until ready to use.

BD CBA Human TGF- β 1 Flex Set Assay Procedure

Following the preparation and dilution of the individual assay components transfer the Standards or samples, Capture Beads, and PE Detection Reagents to the appropriate assay wells or tubes for incubation and analysis. **Please follow the instructions below as they may differ from those provided in the Human Soluble Protein Master Buffer Kit Manual. Investigators should note that in some cases, additional Wash Buffer may be needed, which may be purchased separately (MN 560105).**

Note: Protect Capture Beads and PE Detection Reagents from direct exposure to light.

For Plates:

- Prepare all reagents as described in previous sections before starting the experiment.
- Pre-wet the plate by adding 100 μL of Wash Buffer to each well. To remove the excess volume, apply to vacuum manifold. Do not exceed 10 $^{\circ}$ Hg of vacuum pressure. Aspirate until wells are drained (2 - 10 seconds).
- Vortex the diluted Capture Beads for at least 5 seconds. Add 50 μL of the diluted Capture Beads to each assay well.
- Add 50 μL of standard or samples to the assay wells.
- Mix the plate for 5 minutes using a digital shaker at 500 RPM (do not exceed 600 RPM) and incubate plate for 2 hours at RT.
- Apply the plate to the vacuum manifold and vacuum aspirate (do not exceed 10 $^{\circ}$ Hg of vacuum pressure) until wells are drained (2 - 10 seconds).
- Add 50 μL of the diluted PE Detection Reagent to each assay well.
- Mix the plate for 5 minutes using a digital shaker at 500 RPM and incubate plate at RT for 2 hours.
- Apply the plate to the vacuum manifold and vacuum aspirate (do not exceed 10 $^{\circ}$ Hg of vacuum pressure) until wells are drained (2 - 10 seconds).
- Add 150 μL of Wash Buffer to each assay well.
- Apply the plate to the vacuum manifold and vacuum aspirate (do not exceed 10 $^{\circ}$ Hg of vacuum pressure) until wells are drained (2 - 10 seconds).
- Add 150 μL of Wash Buffer to each assay well. Shake microwell plate on a digital shaker at 500 RPM for 5 minutes to resuspend beads.
- Begin analyzing samples on a flow cytometer.

Note: It is best to analyze samples on the day of the experiment. Prolonged storage of samples, once the assay is complete, can lead to increased background and reduced sensitivity.

For Tubes:

- Prepare all reagents as described in previous sections before starting the experiment.
- Vortex the diluted Capture Beads for at least 5 seconds. Add 50 μL of the diluted Capture Beads to each assay tube.
- Add 50 μL of Standard or sample to each assay tube.
- Mix assay tubes gently and incubate for 2 hours at RT.
- Add 1.0 mL of Wash Buffer to each assay tube and centrifuge at 200 \times g for 5 minutes.
- Carefully aspirate and discard the supernatant from each assay tube leaving approximately 100 μL of liquid in each assay tube. Aspiration should be done as consistently as possible.
- Add 50 μL of the diluted PE Detection Reagent to each assay tube.
- Mix assay tubes gently and incubate for 2 hours at RT.
- Add 1.0 mL of Wash Buffer to each assay tube and centrifuge at 200 \times g for 5 minutes.
- Carefully aspirate and discard the supernatant from each assay tube leaving approximately 100 μL of liquid in each assay tube. Aspiration

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- should be done as consistently as possible.
- Add 300 µL of Wash Buffer to each assay tube. Vortex assay tubes briefly to resuspend beads.
 - Begin analyzing samples on a flow cytometer. It is recommended that each tube be vortexed briefly before analyzing on the flow cytometer.

Note: It is best to analyze samples on the day of the experiment. Prolonged storage of samples, once the assay is complete, can lead to increased background and reduced sensitivity.

The BD CBA Human TGF-β1 Flex Set must be used in conjunction with a BD CBA Human Soluble Protein Master Buffer Kit (Cat. No. 558264, 100 tests, or 558265, 500 tests), a flow cytometer, and the FCAP Array™ Software (Cat. No. 641488). The top standard point for the BD CBA Human TGF-β1 Flex Set will be 10,000 pg/ml. An example standard curve is shown in Figure 1.

Performance

Limit of Detection: The theoretical limit of detection is 14.9 pg/ml and was determined by evaluating the estimated result of the average MFI of the negative control (0 pg/ml, n=30) + 2 standard deviations.

Specificity		Inter-Assay Reproducibility			Intra-Assay Reproducibility		
		Mean (pg/ml)	Standard Deviation	%CV	Mean (pg/ml)	Standard Deviation	%CV
Human TGF β1	Sample 1	145.3	11.7	8%	151.4	8.9	6%
	Sample 2	600.7	29.1	5%	619.3	25.6	4%
	Sample 3	2441.0	66.1	3%	2480.6	154.3	6%

Reproducibility: The intra-assay and inter-assay reproducibility were determined for the BD CBA Human TGF-β1 Flex Set by evaluating ten replicates of three different sample levels (intra-assay) and three replicates of three different sample levels from four separate experiments (inter-assay).

Specificity	Cell Culture Supernatant		Serum		Plasma	
	Average % Recovery	Range	Average % Recovery	Range	Average % Recovery	Range
Human TGF β1	80%	77 - 82%	67%	64 - 71%	13%	8 - 17%

Recovery: Acid treated and diluted Cell culture supernatant, serum, or EDTA-treated plasma were spiked with three different levels of protein. The spiked samples were assayed and the results were compared with expected values. Serum is a pool of 800 - 1000 donors and the plasma was pooled from at least 20 donors. The low recovery suggests that this assay may not be optimal for plasma samples.

Sample Dilution	Cell Culture Supernatant		Serum		Plasma	
	Detected (pg/mL)	% of Expected	Detected (pg/mL)	% of Expected	Detected (pg/mL)	% of Expected
Spiked sample	1943.5	100%	1454.1	100%	405.3	100%
1 : 2	922.9	95%	789.0	109%	289.6	143%
1 : 4	445.0	92%	455.2	125%	219.8	217%

Linearity: Acid treated and diluted Cell culture supernatant, serum or EDTA-treated plasma were spiked with protein and serially diluted. The diluted samples were assayed and the results were compared with the original spiked sample. The poor linearity suggests that this assay may not be optimal for plasma samples.

Product Notices

- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- ProClin is a trademark of Rohm and Haas Company.
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- Warning: CBA lyophilized standard contains 0.02% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).
 Hazard statement: May cause an allergic skin reaction.
 Precautionary statements: Wear protective gloves/eye protection. Wear protective clothing. Avoid breathing mist/vapours/spray. If skin irritation or rash occurs: Get medical advice/attention. IF ON SKIN: Wash with plenty of water. Dispose of contents/container in accordance with local/regional/national/international regulations.

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