



BIS03-EN.01

Bispecific Human CD20 & CD3E Bridging ELISA Kit

Pack Size: 96 tests

Catalog Number: BIS-A003

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

INTENDED USE

Bispecific Human CD20 & CD3E Bridging ELISA Kit is developed for the detection of anti-CD20 & CD3E antibodies in serum and cell culture supernates. It is intended for research use only (RUO).

BACKGROUND

Bispecific antibodies (BsAbs) are engineered immunoglobulins that simultaneously bind two distinct antigens or two different epitopes on the same antigen. Their function depends on precise heavy- and light-chain pairing and molecular architecture, which determine avidity, orientation, and functional potency. By physically bridging immune effector cells to target cells, BsAbs can redirect and amplify immune-mediated cytotoxicity, offering enhanced tumor killing and a reduced likelihood of resistance compared with some monospecific therapies.

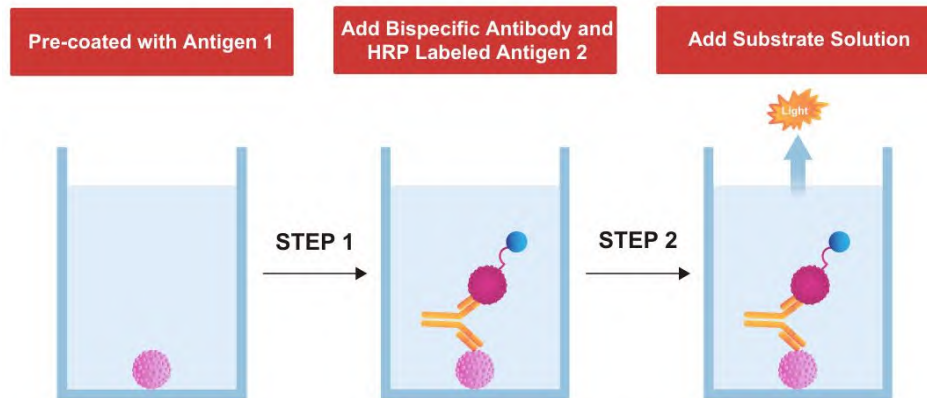
B-lymphocyte antigen CD20 is also known as B-lymphocyte surface antigen B1, Leukocyte surface antigen Leu-16, Membrane-spanning 4-domains subfamily A member 1 and MS4A1, is an activated-glycosylated phosphoprotein expressed on the surface of all B-cells beginning at the pro-B phase (CD45R+, CD117+) and progressively increasing in concentration until maturity. CD20 is expressed on all stages of B cell development except the first and last; it is present from late pro-B cells through memory cells, but not on either early pro-B cells or plasma blasts and plasma cells. CD3E is the epsilon chain of the CD3 complex, a single-pass type I transmembrane glycoprotein containing an Ig-like domain and an ITAM; together with other CD3 chains and the T-cell receptor heterodimers it forms the TCR-CD3 complex that couples antigen recognition to intracellular signaling and is essential for T-cell development and activation. A CD20 & CD3E bridging ELISA evaluates a bispecific molecule's ability to form the ternary complex between CD20 and CD3E, providing a direct measure of the antibody's primary mechanism of action.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of anti-CD20 & CD3E antibodies by employing a standard Bridging-ELISA format. The microplate in the kit has been pre-coated with Human CD20 Protein. First add the standard samples provided in the kit and your samples to the plate, then add the HRP-Human CD3E Protein to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of

anti-CD20 & CD3E antibodies present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of anti-CD20 & CD3E antibodies bound.

FIGURE 1. Bispecific Bridging ELISA Assay Principle



MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
BIS003-C01	Pre-coated Human CD20 Protein Microplate	1 plate	Solid	2-8°C	2-8°C
BIS003-C02	Anti-CD3 & CD20 Antibody Standard	20 µg	Powder	2-8°C	-70°C
BIS003-C03	HRP-Human CD3E Protein	20 µg	Powder	2-8°C, avoid light	-70°C, avoid light
BIS003-C04	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
BIS003-C05	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
BIS003-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
BIS003-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Centrifuge;

37°C Incubator;

10 µL, 200 µL and 1000 µL precision pipettes;

10 µL, 200 µL and 1000 µL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized or distilled water to dilute 10×Washing Buffer;

STORAGE AND VALIDITY INSTRUCTIONS

1. Store the unopened kit at 2-8°C upon receipt.
2. Locate the expiration date on the outer packaging and do not use reagents beyond their expiration date.
3. The opened kit should be stored according to the storage conditions listed in the Components Table. The shelf life of the opened kit is 30 days from the date of opening.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in a 37 °C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.
2. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2 and place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking. The reconstituted stock solutions should be stored at -70°C. It is recommended not to freeze-thaw more than 1 time, the packing specification shall not be less than 5 µg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
BIS003-C02	Anti-CD3 & CD20 Antibody Standard	20 µg	100 µg/mL	200 µL water
BIS003-C03	HRP-Human CD3E Protein	20 µg	100 µg/mL	200 µL water

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of HRP-Human CD3E Protein working fluid:

Dilute HRP-Human CD3E Protein to 0.4 µg/mL with 1×Dilution Buffer. The prepared working fluid should avoid light.

Please prepare it for one-time use only.

1.4 Sample preparation:

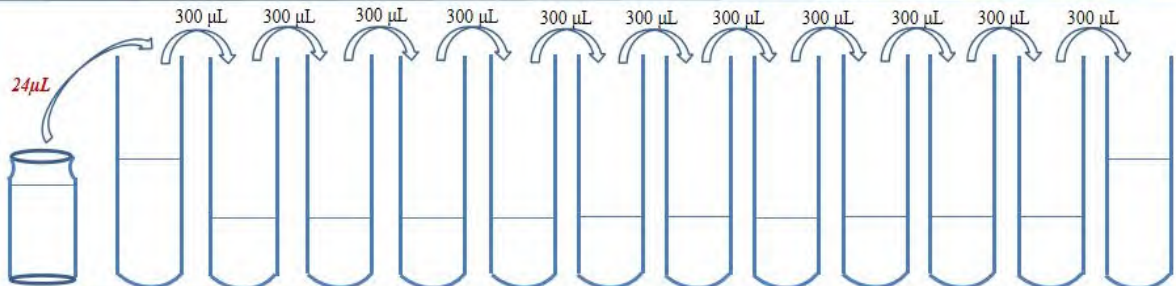
a. If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

b. If the sample to be tested is serum, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

2. Preparation of Standard curve

Make serial dilutions of the Anti-CD3 & CD20 Antibody as a Standard curve with Dilution Buffer as recommended in Figure 2.

FIGURE 2. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Anti-CD3 & CD20 Antibody

Tubes/ Solution Code	Anti-CD3 & CD20 Antibody Standard stock solution	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6	Std.-7	Std.-8	Std.-9	Std.-10	Std.-11	Std.-12
Operating		300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL
Solution Con.	100 µg/mL	4000 ng/mL	2000 ng/mL	1000 ng/mL	500 ng/mL	250 ng/mL	125 ng/mL	62.5 ng/mL	31.25 ng/mL	15.625 ng/mL	7.813 ng/mL	3.906 ng/mL	1.953 ng/mL
Dilution Buffer Vol.		576 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL

3. Add Samples

Add **50 µL** serially diluted **Anti-CD3 & CD20 Antibody Standard** curve and samples to each well. For blank Control wells, please add **50 µL** 1×Dilution Buffer. Then add **50 µL HRP-Human CD3E Protein (dilute to 0.4 µg/mL)** working fluid to each well. Shake gently for 5s to mix. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

Note: a. It is recommended to set double holes for samples and standard curves to be tested.

b. Due to the addition method of 50 µL + 50 µL, the final concentration in the standard curve well differs by two times from the dilution concentration.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 30 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

5. Substrate Reaction

Add 100 µL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at room

temperature for **20 min**, avoid light.

6. Termination

Add 50 μ L **Stop Solution** to each well and tap the plate gently to allow thorough mixing.

Note: The color in the wells should change from blue to yellow.

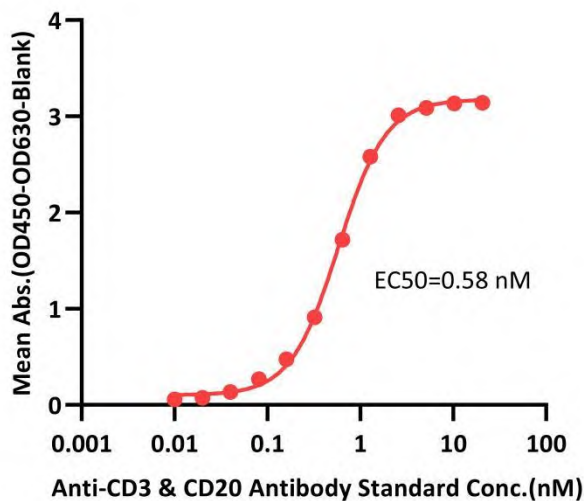
7. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

Note: To reduce the background noise, subtract the value read at OD_{450 nm} with the value read at OD_{630 nm}.

TYPICAL DATA

The following data is for reference only, and the specific OD value may vary depending on different laboratories, experimenters, or equipments.



Anti-CD3 & CD20 Antibody Standard (ng/mL)	Anti-CD3 & CD20 Antibody Standard (nM)	Mean Abs(OD450-630nm)	Mean Abs(OD450-630nm-Blank)
4000	20.619	3.193	3.141
2000	10.309	3.185	3.134
1000	5.155	3.139	3.088
500	2.577	3.062	3.011
250	1.289	2.633	2.581
125	0.644	1.768	1.717
62.5	0.322	0.963	0.911
31.25	0.161	0.526	0.475
15.625	0.081	0.319	0.268
7.813	0.040	0.186	0.134
3.906	0.020	0.125	0.073
1.953	0.010	0.108	0.057
Blank	Blank	0.051	0.000

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balance to room temperature (20 °C -25 °C) before use. If crystals have formed in buffer

solution, warm to room temperature until the crystals have completely dissolved.

5. The kit should be stored at 2°C-8°C.

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again