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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Protein Of Interest (POI): CD3 Bridging Chemiluminescent Assay Kit is an ELISA-based assay designed to analyze the ability of a Bispecific Antibody (BsAbs) to POI and CD3 (cluster of differentiation 3) for screening and profiling applications. This assay allows to study the simultaneous binding to CD3 and to a protein/antigen of interest of bispecific antibodies. The protein of interest **must be provided by the user**. The Protein of Interest (POI): CD3 Bridging Chemiluminescent Assay Kit comes with enough CD3-Containing Detection Reagent, assay buffers and controls (reference capture antigen (BCMA protein) and reference bispecific antibody (targeting BCMA)) for 100 reactions.

This kit provides the user with the flexibility to test their own specific POI, compare variants, or assay several POIs at once for specific determination or epitope mapping, using an optimized protocol and CD3 reagent. The native state of the POI, optimal concentration to be used in plate coating, and ability to be recognized by the test antibodies should be assessed prior the testing with this assay kit.

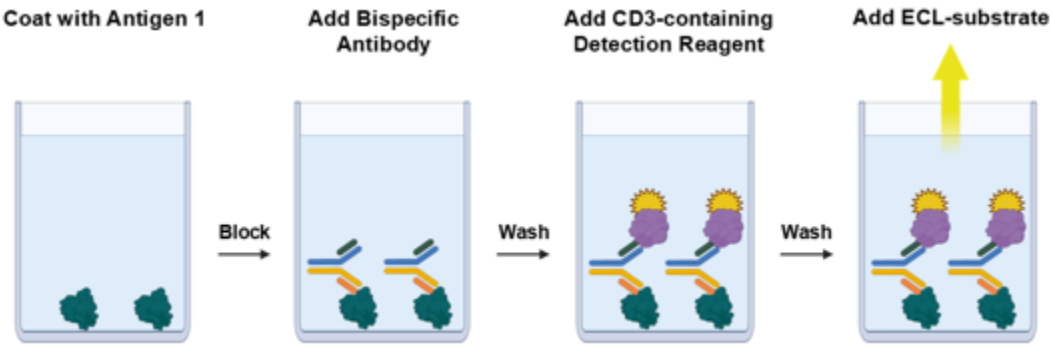

Figure 1: Protein Of Interest (POI): CD3 Bridging Chemiluminescent Assay Kit assay principle.
First a 96-well plate is coated with POI and blocked. Next, bispecific antibodies are added in an optimized assay buffer. This is followed by washing to remove any unbound bispecific antibodies, and the plate is incubated with CD3-Containing Detection Reagent. Finally, ELISA ECL substrate is added and the chemiluminescence generated can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the efficacy of the bridging.

Background

Bispecific antibodies (BsAbs) are antibodies that have binding sites directed against different antigens or different epitopes of the same antigen. They are composed of heavy and light chains, and the appropriate chain matching is required for efficacy. To enhance the chance of chain matching and different applications, more than 30 different technologies have been developed, such as knob-into hole, ART-Ig, BiTE (bispecific cell engager) and others. They have gained attention for the treatment of cancer and other disorders due to their superior cytotoxicity effects and less development of resistance to their use. BsAbs can act as immune cell-cancer cell bridges, enhancing the killing potential of the immune cells. Current BsAbs have a CD3, CD16 or CD47 as one of the binding domains, and target CD19, PD-1, LAG-3, PSMA, mesothelin or other tumor antigens with the other. For instance, B-lymphocyte antigen CD19 (Cluster of Differentiation 19), also known as B-Lymphocyte Surface Antigen B4 and CVID3C, is a transmembrane protein expressed in follicular dendritic cells and all B lineage cells, and is involved in the balance of humoral, antigen-induced and tolerance responses. Its presence in normal and neoplasmic B cells has made it an attractive target in cancer therapy. Blinatumomab is a CD19/CD3 BiTE already

approved by the FDA for use in patients with refractory or relapsed B-cell precursor ALL (acute lymphoblastic leukemia), and now being considered in CLL (chronic lymphocytic leukemia) treatment. The development of BsAbs targeting new targets will advance multiple biomedical research field.

Applications

Study complex formation and screen simultaneously the BsAbs binding affinity towards dual-antigen targets for drug discovery and high throughput screening (HTS) applications.

Note: Suitable for use with human serum, cell culture supernatants, and with purified proteins.

Supplied Materials

Catalog #	Name	Amount	Storage
100689	Reference Bispecific Antibody (Anti-BCMA-Anti-CD3 Bispecific Molecule)*	5 µg	-80°C
79465	Reference Capture Antigen (BCMA, Fc-Fusion, Avi-Tag)*	2 µg	-80°C
82705	CD3-Containing Detection Reagent	6 µl	-80°C
82620	5x PP-02 Buffer	4 ml	-80°C
79743	Blocking Buffer 3	50 ml	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

**The concentration of the proteins is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- POI, to be used for plate coating as the capture target antigen
- Test Samples (biologics expected to target both the POI and CD3) (purified recombinant proteins, human serum, cell culture supernatant)
- PBS Buffer (phosphate buffered saline buffer)
- PBST Buffer (1x PBS with 0.05% Tween-20)
- Microplate reader capable of reading luminescence
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This kit is compatible with up to 1% DMSO.

Assay Protocol

- It is recommended that the binding of the POI to the test antibody be determined in advance, and the coating concentration is optimized. Note that certain coating buffers can stabilize the antigen used to coat the multi-well ELISA plate, maximizing adsorption to the plate and optimizing further interactions with the antibody. PBS and bicarbonate buffer are two of the most used coating buffers when performing ELISA assays. We recommend that the titration of POI is done using these buffers.
- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Negative Control" and "Test Sample" as well as "Blank Reference", "Negative Reference" and "Positive Reference" conditions as additional controls validating assay performance.
- We recommend maintaining the diluted proteins on ice during use.
- Variation in sample collection, processing and storage may cause differences in sample assay results.
- We recommend using Reference Bispecific Antibody (Anti-BCMA-Anti-CD3 Bispecific Molecule) together with the Reference Capture Antigen protein (BCMA, Fc-Fusion, Avi-Tag) as internal control. We recommend running the Reference bsAb at 20X the EC_{50} value shown in the validation data below.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

Step 1: Coat 96-well module

1. Prepare the desired POI at the optimized testing concentration in PBS (50 µl/well).

Note: The native state of POI as well as the optimal concentration of POI to be coated on the plate should be determined prior to performing the assay. Usually 100 ng/well is a typical starting concentration for binding studies.

2. Add 50 µl of diluted POI to the "Test Sample" and "Blank" wells.

3. Thaw Reference Capture Antigen (BCMA) on ice. Briefly spin the tube containing the protein to recover its full content.

4. Dilute Reference Capture Antigen to 2 ng/µl with PBS (50 µl/well).

5. Add 50 µl of diluted Reference Capture Antigen to the "Positive Reference" and "Blank Reference" wells.

6. Add 50 µl of Blocking Buffer 3 to the "Negative Control" and "Negative Reference" wells.

7. Incubate at 4°C overnight (O/N).
8. Wash the plate three times using 200 µl of PBST Buffer per well.
9. Tap the plate onto clean paper towel to remove the liquid.
10. Block the wells by adding 200 µl of Blocking Buffer 3 to every well.
11. Incubate at Room Temperature (RT) for at least 90 minutes.
12. Wash the plate three times using 200 µl of PBST Buffer per well.
13. Tap the plate onto clean paper towel to remove the liquid.

		Reference Controls			Test		
		Blank Reference	**Positive Reference**	**Negative Reference**	**Blank**	**Test Sample**	**Negative Control**
Coating	Diluted POI (pre-optimized concentration)	-	-	-	50 µl	50 µl	-
	Diluted Reference Capture Antigen (2 ng/ µl)	50 µl	50 µl	-	-	-	-
	Blocking Buffer 3			50 µl			50 µl
Incubate O/N at 4°C and wash							
Reaction	1x Assay Buffer	50 µl	-	-	50 µl		
	Test Antibody/Biologic	-	-	-	-	50 µl	50 µl
	Diluted Reference Bispecific Antibody		50 µl	50 µl	-	-	-

Step 2: Bridging reaction

1. Prepare 1x Assay Buffer by diluting 5x PP-02 Assay Buffer 5-fold with distilled water.
2. Thaw Reference Bispecific Antibody (Anti-BCMA-Anti-CD3 Bispecific Molecule) on ice. Briefly spin the tube to recover the full content of the tube.
3. Prepare the Reference Bispecific Antibody by diluting it to 5.7 ng/µl with 1x Assay Buffer (50 µl/well, 100 nM final concentration).
4. Add 50 µl of diluted Control Bispecific Antibody to the “Positive Reference” and “Negative Reference” wells.

5. Prepare the Test Antibody/Biologic (50 µl/well).

   5.1 For a titration of the tested Antibody/Biologic prepare serial dilutions of the tested antibody/biologic in 1x Assay Buffer. The final volume of the reaction is 50 µl.

6. Add 50 µl of diluted Test Antibody/Biologic to the wells labeled "Test Sample" and "Negative Control".

7. Add 50 µl of 1x Assay Buffer to the "Blank" and "Blank Reference" wells.

8. Incubate the plate at RT with slow agitation for 1 hour.

9. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.

10. Thaw CD3 Detection Reagent on ice. Briefly spin the tube containing the protein to recover its full content.

11. Dilute CD3 Detection Reagent 1000-fold with Blocking Buffer 3 (50 µl/well).

12. Add 50 µl of diluted CD3 Detection Reagent to all wells.

13. Incubate the plate at RT with slow agitation for 1 hour.

14. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.

Step 3: Detection

1. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

2. Add 100 µl of mix to every well.

3. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

   Note: The "Blank" or "Negative" value (whatever is higher) should be subtracted from the readings of the Test Sample.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control.

Example Results

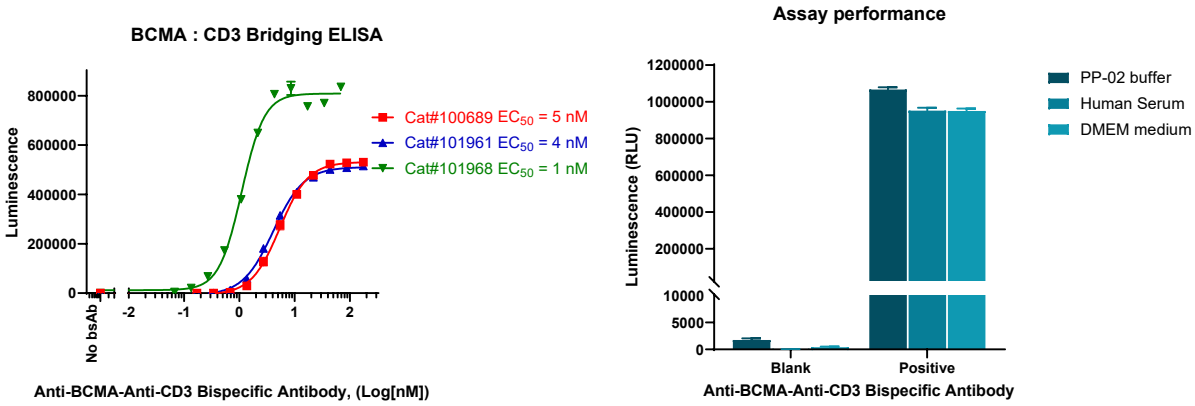

Figure 2. Assay performance and bridging abilities of several bispecific antibodies that target CD3 and BCMA. Right panel: The bridging abilities of several bispecific antibodies targeting BCMA and CD3 (#101968, #100689 and #101961) were determined. Left panel: Effect of cell culture medium or human serum on the bridging efficiency of Anti-BCMA-Anti-CD3 IgG Bispecific Antibody (#101968) was tested. Luminescence was measured using a Bio-Tek microplate reader. Results are presented as total luminescence.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Robinson H., *et al.*, 2018 *Blood* 132 (5): 521-532.
Ma J., *et al.*, 2021 *Front Immunol.* 12:626616.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Bispecific BCMA:CD3 Bridging Chemiluminescence ELISA Kit	82801	96 reactions
Bispecific BCMA:CD3 Bridging Colorimetric ELISA Kit	82807	96 reactions
Bispecific CD19:CD3 Bridging Chemiluminescence ELISA Kit	82764	96 reactions
Bispecific Mesothelin:CD3 Bridging Chemiluminescence ELISA Kit	82830	96 reactions
Bispecific Claudin18.2:CD3 Bridging Chemiluminescence ELISA Kit	82828	96 reactions

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