SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The Bispecific BCMA:CD3 Bridging Colorimetric ELISA Kit is an ELISA designed to analyze the ability of Bispecific Antibodies (BsAbs) to bridge BCMA (B-Cell Maturation Antigen, also known as Tumor necrosis factor receptor superfamily member 17 (TNFRSF17)) and CD3 (cluster of differentiation 3) for screening and profiling applications. This assay kit can determine if an anti-BCMA-anti-CD3 bispecific antibody binds to both targets simultaneously and bridges BCMA to CD3. The Bispecific BCMA:CD3 Bridging Colorimetric ELISA Assay Kit comes with enough recombinant human BCMA (amino acids 20-291), CD3- Containing Detection Reagent, and assay buffer for 100 enzyme reactions. This kit also includes Anti-BCMA-Anti-CD3 Bispecific Antibody as a positive control.

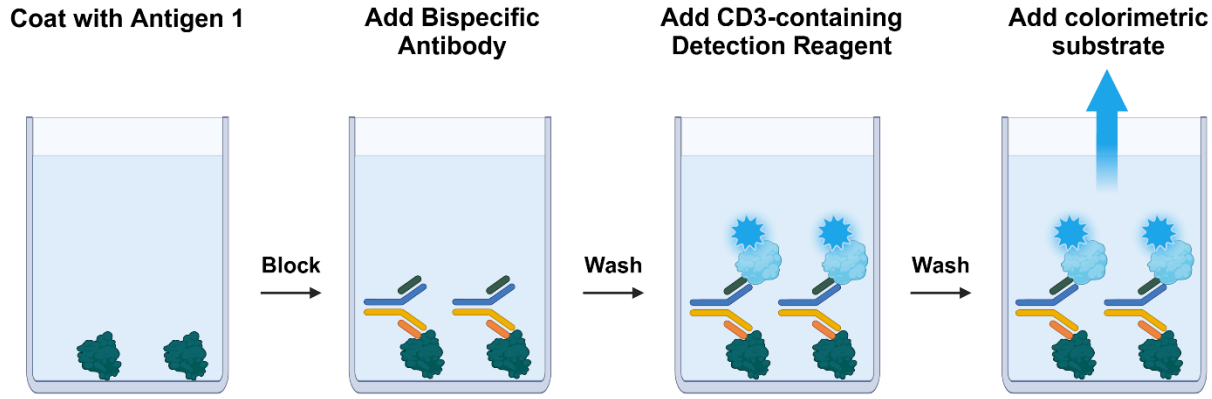

Figure 1: Bispecific BCMA:CD3 Bridging Colorimetric ELISA Assay Kit principle.
A 96-well plate is coated with BCMA protein. After coating, an anti-BCMA-anti-CD3 bispecific antibody is added in an optimized assay buffer. The plate is washed to remove the unbound antibody. The plate is then incubated with CD3-Containing Detection Reagent. Finally, HRP Colorimetric Substrate is added to produce absorbance that can be measured using a UV/Vis spectrophotometer microplate reader. The absorbance signal is proportional to the bridging efficacy.

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. B-cell maturation antigen (BCMA), also known as CD269 or tumor necrosis factor receptor superfamily member 17 (TNFRSF17), is a cell surface receptor of the TNF receptor superfamily that recognizes B-cell activating factor (BAFF). BCMA is preferentially expressed in mature B lymphocytes and on Multiple Myeloma (MM) cells. BCMA is a highly attractive target antigen for immunotherapy because of its restricted expression in nonmalignant tissue but almost universal expression on MM cells. So far FDA has approved two BCMA CAR-T therapies for the treatment of multiple myeloma.

Applications
Study BCMA:CD3 complex formation and assess the binding of BsAbs to their dual-antigen targets simultaneously for drug discovery and high throughput screening (HTS).

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

Supplied Materials

Catalog #	Name	Amount	Storage
79465	BCMA, Fc-Fusion, Avi-Tag*	10 µg	-80°C
100689	Anti-BCMA-Anti-CD3 Bispecific Molecule*	5 µg	-80°C
82705	CD3- Containing Detection Reagent	6 µl	-80°C
82620	5x PP-02 Buffer	4 ml	-20°C
82765	Blocking Buffer 9	50 ml	+4°C
79651	HRP Colorimetric Substrate	10 ml	+4°C
79964	Transparent 96-well plate	1	Room Temp

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

Materials Required but Not Supplied

- Test Samples (purified recombinant proteins, human serum, cell culture supernatant)
- 1x PBS (Phosphate Buffer Saline)
- PBST Buffer (1x PBS with 0.05% Tween-20)
- 2 M sulfuric acid
- UV/Vis spectrophotometer microplate reader capable of reading absorbance
- 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

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control", and "Test Sample" conditions.
- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- Variation in sample collection, processing and storage may cause differences in sample assay results.
- If using human serum or cell culture supernatant we recommend the use of LysA™ Protease Inhibitor Cocktail Kit (#82199) during sample preparation and analysis.
- We recommend using Anti-BCMA-Anti-CD3 Bispecific Molecule (#100689) as internal control. If not running a dose response curve for the control bsAb, we recommend running the Anti-BCMA-Anti-CD3 Bispecific Molecule at 0.1X, 1X and 10X the EC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (https://bpsbioscience.com/serial-dilution-protocol).

Step 1: Coat 96-well plate

Coat the plate one day prior to running your samples.

1. Thaw BCMA on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute BCMA protein to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted BCMA to every well.
4. Incubate at 4°C overnight.
5. Wash the plate three times using 200 µl of PBST Buffer per well.
6. Tap the plate onto a clean paper towel to remove the liquid.
7. Block the wells by adding 200 µl of Blocking Buffer 9 to every well.
8. Incubate at Room Temperature (RT) for at least 90 minutes.
9. Wash the plate three times using 200 µl of PBST Buffer per well.
10. Tap the plate onto a clean paper towel to remove the liquid.

Step 2: Bispecific Bridging

1. Prepare 1x Assay Buffer by diluting 5x PP-02 Assay Buffer 5-fold with distilled water.
2. Prepare the diluted Anti-BCMA-Anti-CD3 Bispecific Molecule control (Control BsAb) (50 µl/well).

    2.1 Thaw Anti-BCMA-Anti-CD3 Bispecific Molecule on ice. Briefly spin the tube to recover the full content of the tube.

2.2 For a dose response curve prepare a serial dilution starting at 10 ng/µl of the Anti-BCMA-Anti-CD3 Bispecific Molecule with 1x Assay Buffer (50 µl/well).

3. Add 50 µl of diluted Anti-BCMA-Anti-CD3 Bispecific Molecule to the "Positive Control" wells.

4. Prepare serial dilutions of the biologic of interest (test sample) with 1x Assay Buffer at the desired final concentrations (50 µl/well).

5. Add 50 µl of each test sample to the wells labeled "Test Sample".

6. Add 50 µl of 1x Assay Buffer to "Blank" wells.

	Blank	**Positive Control**	**Test Sample**
1x Assay Buffer	50 µl	-	-
Test Sample	-	-	50 µl
Control BsAb	-	50 µl	-
Total	**50 µl**	**50 µl**	**50 µl**

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

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

   Note: Additional blocking may improve S/N ratio. If necessary, block by adding 200 µl of Blocking Buffer 9 to every well for 10 min.

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

10. Dilute the CD3-Containing Detection Reagent 1000-fold with Blocking Buffer 9 (50 µl/well).

11. Add 50 µl of diluted CD3-Containing Detection Reagent to all wells.

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

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

Step 3: Detection

1. Add 100 µl of the colorimetric HRP substrate to each well.

2. Incubate the plate at RT until blue color is developed in the "Positive Control" wells.

Note: It normally takes 10-15 minutes to fully develop the color. However, the optimal incubation time may vary and should be determined empirically by the user. If color is intense *the plate can be read right away at*

650 nm without adding 2 M sulfuric acid (see below). To increase the Signal-to-Background ratio proceed to the next step.

3. Add 100 µl of 2 M sulfuric acid to each well.

4. Read the absorbance at 450 nm using a UV/Vis spectrophotometer microplate reader.

5. The "Blank" value should be subtracted from all other values.

Example Results

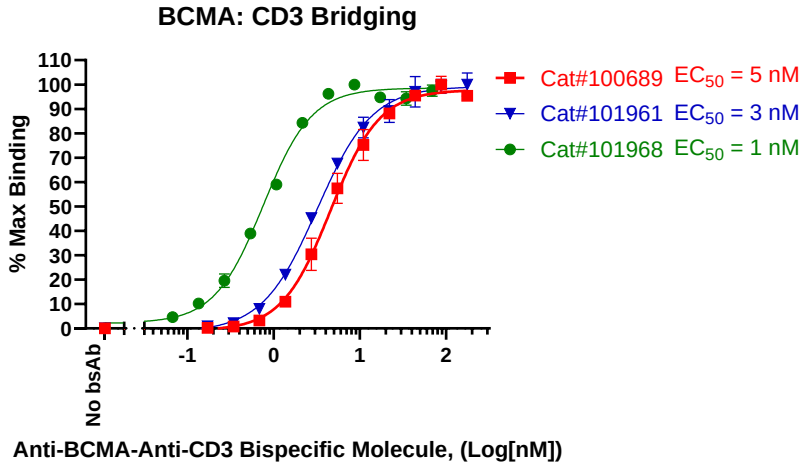

Figure 2. Simultaneous binding abilities of different Anti-BCMA-Anti-CD3 Bispecific Antibodies to their dual-antigen targets.
The bridging abilities of various BsAb targeting BCMA and CD3 (#101968, #100689 and #101961) were validated using Bispecific BCMA:CD3 Bridging Colorimetric ELISA Assay Kit. Absorbance was measured using a Bio-Tek microplate reader. Results are presented as a percentage of bridging in which the maximal binding is set to 100%.

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

References

Ma J., *et al*., 2021 *Front Immunol.* 12:626616.
Ghosh A, *et al.*, 2017 *Leuk. Lymphoma.* 2017; 6: 1-12
Sanchez E., *et al.*, 2018 *Expert Rev Mol Diagn.* 2018; 7: 1-11.
Sohail A., *et al.*, 2018 *Immunotherapy.* 10(4): 265-282.

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*
Bispecifc BCMA:CD3 Bridging Chemiluminescence ELISA Kit	82801	96 reactions
Anti-BCMA CAR-T Cells	78660	1 vial/ 5 vials
BCMA CHO Recombinant Cell Line (High or Low Expression)	79500	2 vials
CD19/ BCMA/ Firefly Luciferase – CHO Recombinant Cell Line	78030	2 vials
BCMA Knockout RPMI-8226 Cell Line	82659	2 vials
Firefly Luciferase BCMA Knockout RPMI-8226 Cell Line	82689	2 vials

Version 012125