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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 Mesothelin:CD3 Bridging Colorimetric ELISA Kit is an ELISA designed to analyze the ability of Bispecific Antibodies (BsAbs) to bridge Mesothelin (also known as MSLN, MPF, SMRP) and CD3 (cluster of differentiation 3) for screening and profiling applications. This assay kit can determine if an anti-Mesothelin-anti-CD3 bispecific antibody binds to both targets simultaneously and bridges Mesothelin to CD3. The Bispecific Mesothelin:CD3 Bridging Colorimetric ELISA Assay Kit comes with enough recombinant human Mesothelin (amino acids 296–580), CD3-Containing Detection Reagent, and assay buffer for 100 enzyme reactions. This kit also includes Anti-Mesothelin-Anti-CD3 Bispecific Antibody as a positive control.

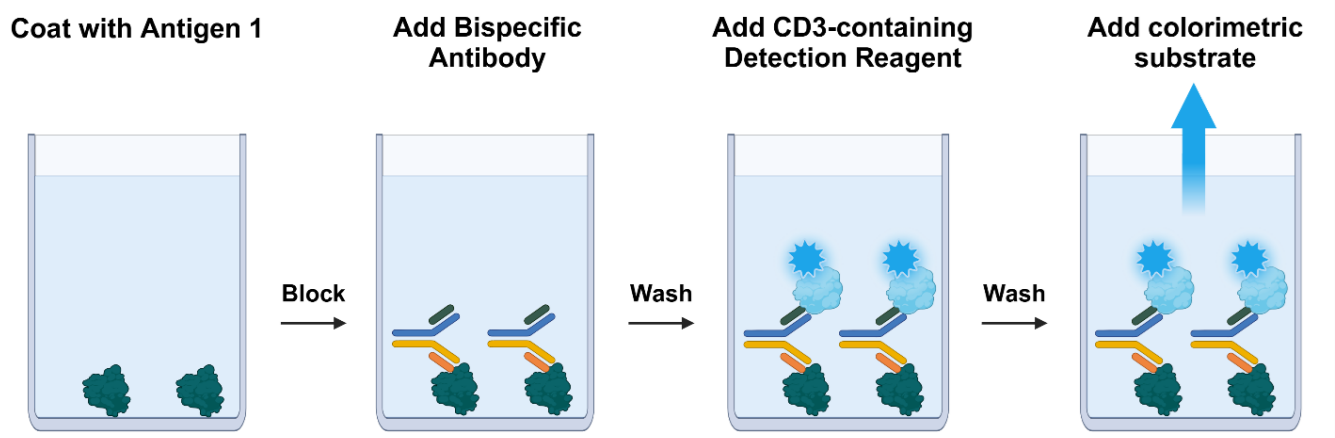

Figure 1: Bispecific Mesothelin:CD3 Bridging Colorimetric ELISA Assay Kit principle.
A 96-well plate is coated with Mesothelin protein. After coating, an anti-Mesothelin-anti-CD3 bispecific antibody is added in an optimized assay buffer. The plate is washed to remove the unbound antibody. The plate is 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. Mesothelin (MSLN) is a glycophosphatidylinositol (GPI) linked cell-surface protein that is produced as a ~70 kDa precursor protein and cleaved by Furin protease to generate the ~40 kDa mature form. MSLN is frequently overexpressed in mesothelioma, ovarian, pancreatic, and non-small cell lung cancers, while its expression in normal tissues is restricted to the mesothelial lining. MSLN is a tumor-associated antigen and has been an attractive target for targeted immunotherapy approaches, including drug-conjugated antibodies and chimeric antigen receptor T cells (CAR-T Cells).

Applications

Study Mesothelin: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
100290	Mesothelin, Avi-His-Tag*	10 µg	-80°C
101621	Anti-Mesothelin-Anti-CD3 Bispecific Antibody*	10 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 microtiter 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)
- 2M 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-Mesothelin-Anti-CD3 Bispecific Antibody (#101621) as internal control. If not running a dose response curve for the control bsAb, we recommend running the Anti-Mesothelin-Anti-CD3 IgG format Bispecific Antibody 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 Mesothelin on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute Mesothelin protein to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted Mesothelin 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-Mesothelin-Anti-CD3 Bispecific Antibody control (Control BsAb) (50 µl/well).

    2.1 Thaw Anti-Mesothelin-Anti-CD3 Bispecific Antibody 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 40 ng/µl of the Anti-Mesothelin-Anti-CD3 Bispecific Antibody with 1x Assay Buffer (50 µl/well).

3. Add 50 µl of diluted Anti-Mesothelin-Anti-CD3 Bispecific Antibody 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

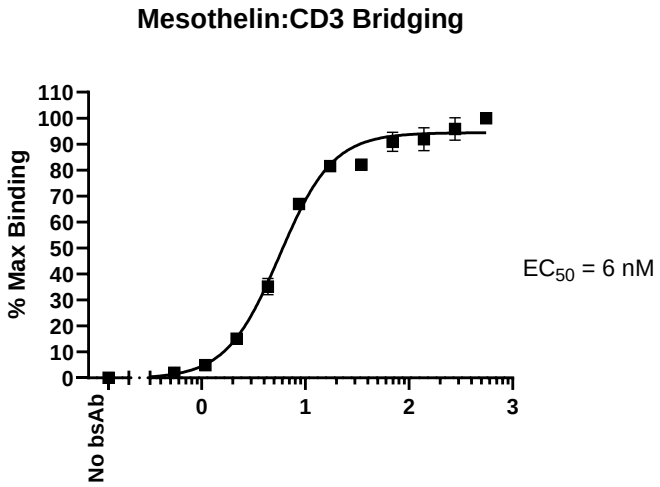

Figure 2. Simultaneous binding ability of Anti-Mesothelin-Anti-CD3 Bispecific Antibody to its dual-antigen targets.
The bridging ability of BsAb targeting Mesothelin and CD3 (#101621) was validated using Bispecific Mesothelin:CD3 Bridging Colorimetric ELISA Assay Kit. Absorbance was measured using a 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.
Asgarov K., *et al.*, 2017 *MAbs* 9(3): 567–577.
Ye L., *et al.*, 2019 *Experimental and Therapeutic Medicine* 17: 739-747.

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 Mesothelin:CD3 Bridging Chemiluminescence ELISA Kit	82830	96 reactions
Anti-Mesothelin CAR-T Cells	78729	1 vial
Anti-Mesothelin CAR Lentivirus (P4 ScFv-CD8-4-1BB-CD3ζ)	78703	50 µl
Human Mesothelin – CHO-K1 Recombinant Cell Line	78132	2 vials
Bispecific CD19:CD3 Bridging Chemiluminescence ELISA Kit	82764	96 reactions
Bispecific BCMA :CD3 Bridging Chemiluminescence ELISA Kit	82801	96 reactions

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