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- 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 Bispecific Claudin-18.2:CD3 Bridging Chemiluminescence ELISA Kit is an ELISA designed to analyze the ability of Bispecific Antibodies (BsAbs) to bridge Claudin-18.2 (Claudin-18, Isoform 2) and CD3 (cluster of differentiation 3) for screening and profiling applications. This assay kit can determine if an anti-Claudin-18.2-anti-CD3 bispecific antibody binds to both targets simultaneously and bridges Claudin-18.2 to CD3. The Bispecific Claudin-18.2:CD3 Bridging Chemiluminescence ELISA Assay Kit comes with enough recombinant human Claudin-18.2, CD3-Containing Detection Reagent, and assay buffer for 100 enzyme reactions. This kit also includes Anti-Claudin-18.2-Anti-CD3 Bispecific Antibody as a positive control.

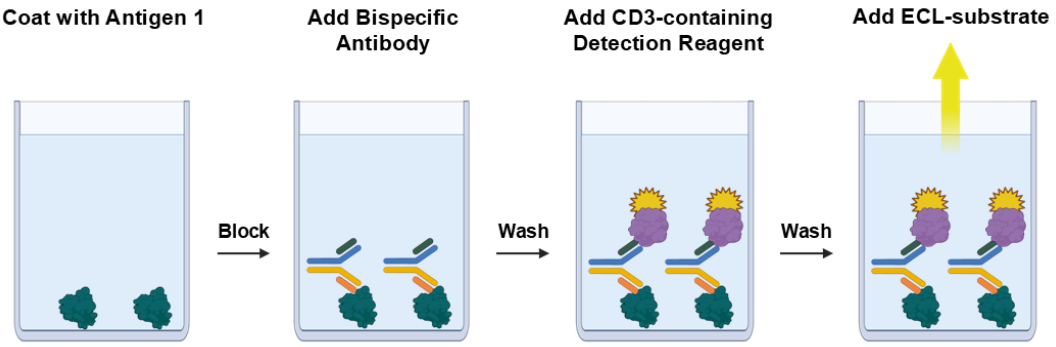

Figure 1: Bispecific Claudin-18.2:CD3 Bridging Chemiluminescence ELISA Assay Kit principle.
A 96-well plate is coated with Claudin-18.2 protein. After coating, an anti-Claudin-18.2-anti-CD3 bispecific antibody is added in an optimized assay buffer. The plate is washed to remove the unbound antibody. The plate is then incubated with CD3-Containing Detection Reagent. Finally, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence 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, Claudin-18.2 or other tumor antigens with the other. Claudin-18.2 is highly expressed in gastric and pancreatic adenocarcinoma and may be involved in tumor development and progression. Due to the morphological changes and epithelial-mesenchymal transition occurring upon cancer progression, the extracellular loops of claudin-18.2 become exposed and available for antibody binding. These biological characteristics suggest that it is an ideal target for therapy and have led to the further development of monoclonal antibodies against claudin-18.2, such as Zolbetuximab.

Applications
Study Claudin-18.2:CD3 complex formation and assess the simultaneously binding of BsAbs to their dual-antigen targets 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
101570	Claudin-18 Isoform 2, FLAG-Tag*	10 µg	-80°C
101541	Anti-Claudin-18 Isoform2-Anti-CD3 IgG 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
	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
79670	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

- Test Samples (purified recombinant proteins, human serum, cell culture supernatant)
- 1x PBS (Phosphate Buffer Saline)
- 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

- 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-Claudin-18 Isoform2-Anti-CD3 IgG Bispecific Antibody (#101541) as internal control. If not running a dose response curve for the control bsAb, we recommend running the Anti-Claudin-18.2-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 Claudin-18.2 on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute Claudin-18.2 protein to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted Claudin-18.2 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-Claudin-18 Isoform2-Anti-CD3 IgG Bispecific Antibody control (Control BsAb) (50 µl/well).

2.1 Thaw Anti-Claudin-18 Isoform2-Anti-CD3 IgG 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-Claudin-18 Isoform2-Anti-CD3 IgG Bispecific Antibody with 1x Assay Buffer (50 µl/well).

3. Add 50 µl of diluted Anti-Claudin-18 Isoform2-Anti-CD3 IgG 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. 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.

4. The “Blank” value should be subtracted from all other values.

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 assay without enzyme (typically we set this value as 100).

Example Results

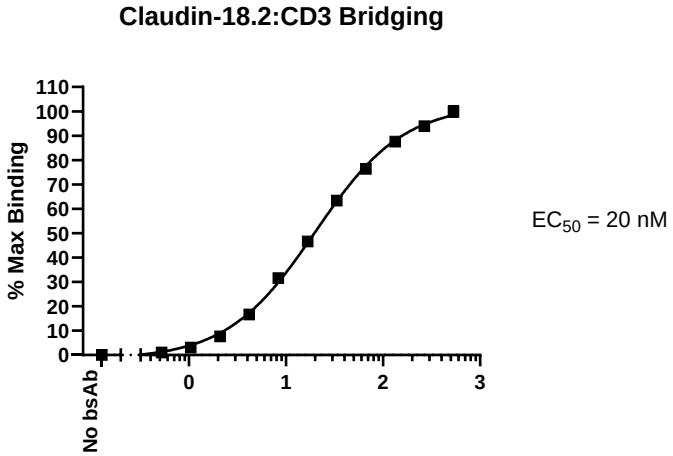

Figure 2. Simultaneous binding ability of Anti-Claudin-18 Isoform2-Anti-CD3 IgG Bispecific Antibody to its dual-antigen targets.
The bridging ability of BsAb targeting Claudin-18.2 and CD3 (#101541) was validated using Bispecific Claudin-18.2:CD3 Bridging Chemiluminescence ELISA Assay Kit. Luminescence 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.

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 Claudin-18.2:CD3 Bridging Colorimetric ELISA Kit	82829	96 reactions
Bispecific CD19:CD3 Bridging Chemiluminescence ELISA Kit	82764	96 reactions
Bispecific BCMA :CD3 Bridging Chemiluminescence ELISA Kit	82801	96 reactions
Claudin-18 Isoform 2 CHO Cell Line (High, Medium, or Low Expression)	78533	2 vials
Anti-Claudin-18 Isoform 2 Antibody, FITC-Labeled	101866	25 µg/ 100 µg
Anti-Claudin-18 Isoform 2 IgG Antibody	101564	50 µg/ 100 µg

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