# CD155:CD96 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit

#82532
96 reactions

**Description**

The CD155:CD96 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is an ELISA-based assay designed for screening and profiling molecules that block the binding between CD155 (cluster of differentiation 155) and CD96 (cluster of differentiation 96). This kit comes in a convenient 96-well format, with enough recombinant human biotin-labeled CD96 (amino acids 22-503), CD155 (amino acids 27-343), streptavidin-labeled HRP, and assay buffer for 100 reactions.

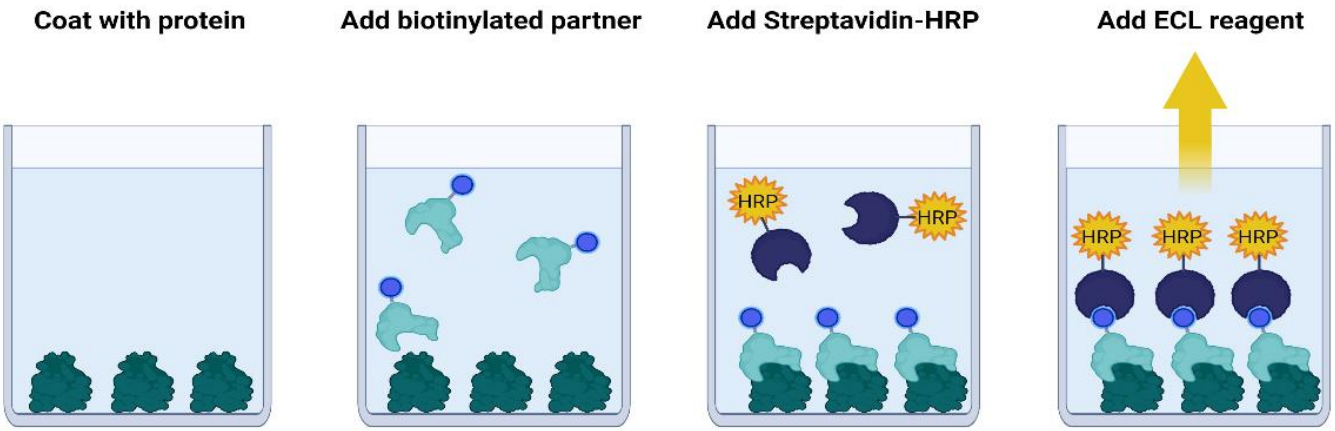


*Figure 1: Illustration of the mechanism of CD155: CD96 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.*

A 96-well plate is coated with CD155 protein. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. After incubation with Biotin-CD96, the plate is washed and Streptavidin-HRP is added. The ELISA ECL substrate is added, and the resulting signal can be measured using a chemiluminescence microplate reader. The chemiluminescence signal is proportional to the binding of CD155 to CD96.

**Background**

CD96, also known as Tactile (T cell activation, increased late expression), is an immune checkpoint receptor of the immunoglobulin superfamily of proteins found in T and NK cells. Other proteins of the same superfamily include CD226 (also known as DNAM-1) and TIGIT (T cell immunoglobulin and ITM domain), with CD96 and TIGIT acting as inhibitory receptors and CD226 as a co-stimulatory receptor. CD96 binds to CD155, competing with CD226. CD96 plays a role in NK-cell adhesion to target cells, inhibiting NK cell cytokine release. It also plays an inhibitory role in Th9 cells and is a cancer stem cell marker. An imbalance in CD155/CD96 and CD155/CD226 can lead to lack of or to excessive immune responses. In LUAD (lung adenocarcinoma) the levels of CD96 in $CD8^+$ T cells are elevated compared to healthy individuals, possibly promoting a hypo-immune response. CD96, together with TIGIT, has become a widely researched target in immunotherapy, and blockage of CD96 binding to CD155 combined with other therapies, such as anti-PD-1 (programmed death protein 1) or doxorubicin chemotherapy, has shown great promise. The development of strategies to block CD96 inhibitory immune responses will continue to be an active area de research, opening new avenues of treatment for cancer patients.

**Application(s)**

Screen or titrate small molecule inhibitors or antibodies that block CD155 binding to CD96 for drug discovery and high-throughput screening (HTS) applications.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| | CD96, His-Tag, Avi-Tag, Biotin-Labeled* | 10 µg | -80°C |
| 79063 | CD155, Fc-Avi-Tag* | 2 x 25 µg | -80°C |
| | 5x PP-02 Buffer | 4 ml | -20°C |
| 79728 | Blocking Buffer 2 | 40 ml | +4°C |
| 79742 | Streptavidin-HRP | 10 µl | +4°C |
| 79670 | ELISA ECL Substrates A (translucent bottle) | 6 ml | Room Temp |
| | ELISA ECL Substrates B (brown bottle) | 6 ml | Room Temp |
| 79699 | White 96-well microplate | 1 | Room Temp |

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

**Materials Required but Not Supplied**

- 1x PBS Buffer (Phosphate Buffer Saline, pH 7.4)
- PBS-T Buffer (1x PBS buffer with 0.05% Tween-20)
- Microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

**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**

The DMSO concentration in the final reaction should be ≤1%.

**Assay Protocol**

- All samples and controls should be tested in duplicate.
- The assay should include “Non-Coated Control”, “Blank”, “Positive Control” and “Test Compound” wells.
- We recommend pre-incubating antibodies or protein inhibitors with the target protein prior to the addition of the binding partner.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.
- We recommend using Anti-CD96 Neutralizing Antibody (#102141) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the $IC_{50}$ value shown in the validation data below.

- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

**Step 1 - Plate coating with CD155**

Coat the plate one day prior to running your samples.

1. Thaw **CD155** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **CD155** protein to 10 ng/µl in PBS (50 µl/well).
3. Add 50 µl of diluted **CD155** protein solution to each well, except "Non-Coated Control" wells.
4. Add 50 µl of PBS to the "Non-Coated Control" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times with 150 µl of PBS-T Buffer per well.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Add 100 µl of Blocking Buffer 2 to every well.
9. Incubate for 90 minutes at Room Temperature (RT) with gentle agitation.
10. Tap the plate onto clean paper towel to remove the liquid.
11. Start your testing immediately.

**Step 2: Assessment of inhibition/blocking of CD155 binding to CD96 by blocking molecules.**

1. Prepare **1x PP-02 Buffer** by diluting 5-fold the **5x PP-02 Buffer** with distilled water.
2. Add 20 µl of 1x PP-02 Buffer to "Non-Coated Control", "Positive Control" and "Test Compound" wells.
3. Add 45 µl of 1x PP-02 Buffer to "Blank" wells.
4. Prepare the Test Compound (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   4.1 If the Test Compound is water-soluble, prepare serial dilutions in 1x PP-02 Buffer at concentrations 10- fold higher than the desired final concentrations.

   **OR**

4.2 If the Test Compound is soluble in DMSO, prepare the compound in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in 1x PP-02 Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

Using 1x PP-02 Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

For positive and negative controls, prepare 10% DMSO in 1x PP-02 Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

*Note: The final concentration of DMSO in the assay should not exceed 1%.*

5. Add 5 µl of the test compound to each well designated "Test Compound".
6. Add 5 µl of Diluent Solution to the "Blank", "Non-Coated Control" and "Positive Control" wells.
7. Incubate the plate for 15-30 minutes at RT with gentle agitation.
8. Thaw the **CD96-Biotin** on ice. Briefly spin the tube to recover the full content.
9. Dilute **CD96-Biotin** to 4 ng/µl with 1x PP-02 Buffer (25 µl/well).
10. Add 25 µl of diluted **CD96-Biotin** to the "Non-Coated Control, "Positive Control," and "Test Compound" wells.
11. Incubate the plate at RT for 1 hour with gentle agitation.

| | **Blank** | **Non-Coated Control** | **Positive Control** | **Test Compound** |
|---|---|---|---|---|
| PP-02 Buffer | 45 µl | 20 µl | 20 µl | 20 µl |
| Test Compound | - | - | - | 5 µl |
| Diluent Solution | 5 µl | 5 µl | 5 µl | - |
| Pre-incubate 15-30 minutes at RT | | | | |
| Diluted CD96-Biotin (4 ng/µl) | - | 25 µl | 25 µl | 25 µl |
| **Total** | **50 µl** | **50 µl** | **50 µl** | **50 µl** |

12. Wash the plate three times with 150 µl of PBS-T Buffer per well.
13. Tap the plate onto clean paper towel to remove the liquid.
14. Block by adding 100 µl of Blocking Buffer 2 to every well and incubate for 10 minutes at RT.
15. Tap the plate onto clean paper towel to remove the liquid.

**Step 3: Detection**

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 2 (50 µl/well).
2. Add 50 µl of diluted **Streptavidin-HRP** to each well.
3. Incubate the plate for 1 hour at RT with gentle agitation.
4. Wash the plate three times with 150 µl of PBS-T Buffer per well.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
7. Add 100 µl of mix to each well.

   *Note: Discard any unused chemiluminescent mix after use.*
8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.
9. The "Blank" value should be subtracted from all readings.

**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).

**Validation Data**

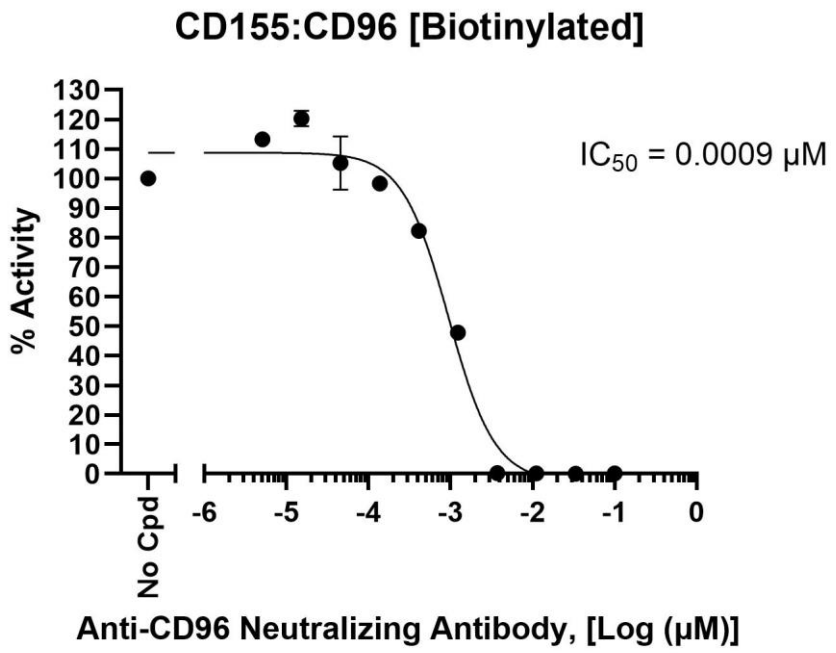


*Figure 2. Inhibition of binding of CD155 to CD96 by Anti-CD96 Neutralizing Antibody.*
CD155:CD96 binding was measured in the presence of increasing concentrations of Anti-CD96 Neutralizing Antibody (#102141). Results are expressed as percent of activity, in which the binding measured in the absence of inhibitor is set to 100%.

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

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

**References**
Zhang H., *et al.*, 2020 *Cellular & Molecular Immunology* 18:1575-1577.
Liu F., *et al.*, 2020 *Scientific Reports* 10:10768.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| CD226:CD155 Homogeneous Assay Kit | 72052 | 384 reactions |
| TIGIT:CD155 Homogeneous Assay Kit | 72029 | 384 reactions |
| CD155:TIGIT[Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit | 82526 | 96 reactions |
| CD155 (PVR) – HEK293 Recombinant Cell Line | 60537 | 2 vials |
| CD96, Fc-Fusion, Avi-Tag (Human) Recombinant | 71265 | 25 µg/100 µg |

| | | |
|---|---|---|
| CD96, Fc-Fusion, Avi-Tag, Biotin Labeled (Human) Recombinant | 71280 | 25 µg/50 µg |

*Version 051624*