Description

The CD112R:CD112 [Biotinylated] Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling of inhibitors or neutralizing antibodies able to block the interaction between CD112R (cluster of differentiation 112 receptor) and CD112. This kit comes in a convenient 96-well format, with enough biotin-labeled CD112 (amino acids 32-360), purified CD112R (amino acids 100-330), streptavidin-labeled HRP, and assay buffer for 100 binding reactions.

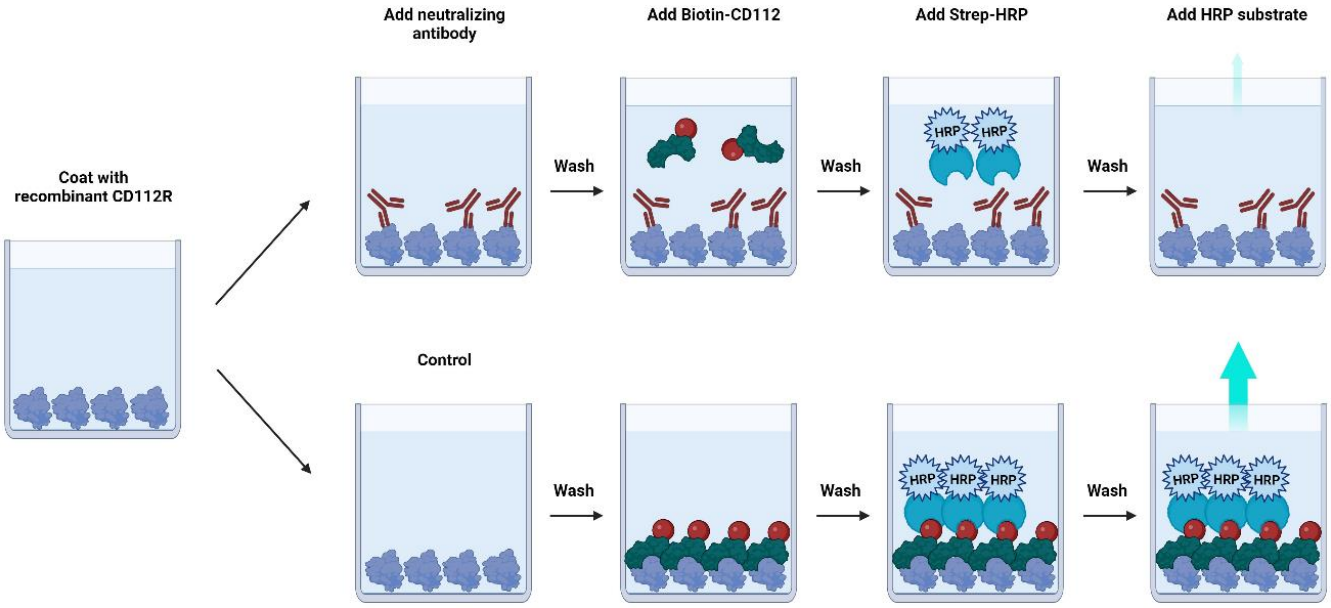

Figure 1: CD112R:CD112 [Biotinylated] Inhibitor Screening Colorimetric Assay Kit schematic.

The key to this kit is the high sensitivity of detection of biotin-labeled CD112 by streptavidin-HRP. CD112R is coated on a 96-well plate. After blocking, the protein is pre-incubated with the inhibitor or neutralizing antibody. Upon subsequent incubation with Biotin-CD112, the plate is treated with Streptavidin-HRP followed by addition of a colorimetric HRP substrate to produce color, which can be quenched and measured using a UV/Vis microplate reader. Absorbance values are proportional to the binding of CD112R to CD112.

Background

CD112 (Poliovirus receptor-related 2, PVRL2), widely expressed on antigen presenting cells and tumor cells, is the high affinity ligand of CD112R (Poliovirus receptor related immunoglobulin domain containing, PVRIG). CD112R is found in NK and T cells, and in T cells it is found particularly in $CD8^+$ T cells. High levels of this protein are found in NK, $CD8^+$ and $CD4^+$ T cells of patients with kidney, ovary, lung, prostrate and endometrium cancer and acute myeloid leukemia (AML). CD112 has also been linked to tumor angiogenesis, growth and metastasis. It has a wider pattern of expression, being also present in endothelial cells, neurons and fibroblasts. CD112-CD112R interaction is a positive immune checkpoint that enhances human T cell response and has emerged as an attractive therapeutic target for oncology. TIGIT (T cell immunoreceptor with Ig and ITIM domains) and CD226 (also called DNAM-1, DNAX Accessory Molecule-1) also bind CD112. The development of inhibitors able to prevent the interaction between CD112R-CD112 alone or in combination with blockage of TIGIT-CD155/CD112 interaction may prove beneficial in cancer therapy.

Application(s)
Screening inhibitors or neutralizing antibodies that block CD112R binding to CD112.

Supplied Materials

Catalog #	Name	Amount	Storage
79116	CD112R (PVRIG), Fc-Fusion, Avi-Tag (Human) HiP™*	2 x 5 µg	-80°C
71234	CD112 (PVRL2), His Avi-Tag, Biotin-labeled (Human) HiP™*	2 x 5 µg	-80°C
79311	3X Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79742	Streptavidin-HRP	5 µl	+4°C
79651	HRP Colorimetric Substrate	10 ml	+4°C
79964	Clear 96-well microplate	1	Room Temp

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

Materials Required but Not Supplied

- PBS (Phosphate Buffered Saline)
- 1N HCl (aqueous)
- Rotating or rocker platform
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at λ=450 nm

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 “Non-Coated Condition”, “Blank”, “Positive Control” and “Test Inhibitor”/“Test Antibody” wells.
- We recommend preincubating antibodies or blockers 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 maintaining the diluted proteins on ice during use.

- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Anti- PVRIG Neutralizing Antibody (BPS Bioscience #101712) as internal control. If not running a dose response curve for the control antibody, we recommend running the control antibody at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1 - Plate coating with CD112R (PVRIG) protein.
Coat the plate one day prior to running your samples in the assay test.

1. Thaw **CD112R (PVRIG)** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **CD112R (PVRIG)** protein to 2 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **CD112R (PVRIG)** protein solution to each well.
4. Add 50 µl of PBS to "Non-Coated Condition" wells.
5. Incubate at 4°C overnight.
6. Prepare **1x Immuno Buffer 1** by diluting 3-fold **3x Immuno Buffer 1** with distilled water.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Wash each well with 100 µl of 1x Immuno Buffer 1.
9. Tap the plate onto clean paper towel to remove the liquid.
10. Repeat steps 8 and 9 twice more.
11. Add 100 µl of Blocking Buffer 2 to every well.
12. Incubate for 1 hour at Room Temperature (RT) with gentle agitation.
13. Tap the plate onto clean paper towel to remove the liquid.
14. Start your assay test immediately.

Step 2.1: Assay for detection of anti-CD112R (PVRIG) antibody inhibition or blocking activity.

1. Prepare a serial dilution of **anti-CD112R (PVRIG)** antibody or blocker in Blocking Buffer 2 at the desired concentrations (50 µl/well).
2. Add 50 µl of the diluted antibody or blocker to the "Test Antibody" wells.
3. Add 100 µl of Blocking Buffer 2 to the "Blank" wells.
4. Add 50 µl of Blocking Buffer 2 to the "Positive Control" wells.

5. Incubate the plate for 30 minutes (up to 1 hour) at RT with gentle agitation.
6. Thaw **CD112 (PVRL2)-Biotin** on ice. Briefly spin the tube to recover the full content.
7. Dilute **CD112 (PVRL2)-Biotin** to 2 µg/ml in Blocking Buffer 2 (50 µl/well).
8. Add 50 µl of diluted **CD112 (PVRL2)-Biotin** to the "Test Inhibitor" and "Positive Control" wells.
9. Incubate the plate at RT for 1 hour with gentle agitation.

	Blank	**Positive Control**	**Test Antibody**
Blocking Buffer 2	100 µl	50 µl	-
Test Antibody	-	-	50 µl
Diluted CD112 (PVRL2)-Biotin (2 µg/ml)	-	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

10. Wash the plate three times with 1x Immuno Buffer 1.
11. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 2 (50 µl/well).
12. Add 50 µl of the diluted Streptavidin-HRP to each well.
13. Incubate the plate for 30 minutes at RT with gentle agitation.
14. Wash the plate three times with 100 µl of 1x Immuno Buffer 1.
15. Prepare 1M HCl (aqueous) (100 µl/well). This is the **Stop Solution**.
16. Add 100 µl of **Colorimetric HRP Substrate** to each well.
17. Incubate the plate at RT until the 'Positive Control' wells' solution becomes blue.

    Note: This usually takes 1-5 minutes. The optimal incubation time may vary and should be determined empirically by the user. It is recommended that the reaction be stopped when the "Positive Control" wells are lower than ~1.0 absorbance at 450 nm (preferably ~0.6).

18. Add 100 µl of Stop Solution to every well. The blue colored solution will turn yellow.
19. Read the absorbance at λ=450 nm using an UV/Vis spectrophotometer microplate reader.

Step 2.2: Assay for detection of small molecule inhibition or blocking activity.

1. Prepare the test inhibitor (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.

1.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water at concentrations 10-fold higher than the desired final concentrations.

For controls use distilled water (Diluent Solution).

OR

1.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

Using distilled water 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 distilled water (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%.

2. Add 5 µl of diluted Test Inhibitor to each well labeled "Test Inhibitor".
3. Add 5 µl of the Diluent Solution to the "Positive Control" and "Blank" wells.
4. Thaw **CD112 (PVRL2)**-**Biotin** on ice. Briefly spin the tube to recover the full content.
5. Dilute CD112 (PVRL2)-Biotin to 2 µg/ml in Blocking Buffer 2 (20 µl/well).
6. Add 20 µl of diluted CD112 (PVRL2)-Biotin to the wells labeled "Test Inhibitor" and "Positive Control".
7. Add 25 µl of Blocking Buffer 2 to the "Test Inhibitor" and "Positive Control" wells.
8. Add 45 µl of Blocking Buffer 2 to the "Blank" wells.

	Blank	**Positive Control**	**Test Inhibitor**
Blocking Buffer 2	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted CD112 (PVRL2)-Biotin (2 µg/ml)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

9. Incubate the plate at RT for 1 hour with gentle agitation.
10. Wash the plate three times with 100 µl of 1x Immuno Buffer 1.

11. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 2 (50 µl/well).
12. Add 50 µl of the diluted Streptavidin-HRP to each well.
13. Incubate the plate for 30 minutes at RT with gentle agitation.
14. Wash the plate three times with 1x Immuno Buffer 1.
15. Prepare 1M HCl (aqueous) (100 µl/well). This is the **Stop Solution**.
16. Add 100 µl of **Colorimetric HRP Substrate** to each well.
17. Incubate the plate at RT until the 'Positive Control' wells' solution becomes blue.

    Note: This usually takes 1-5 minutes. The optimal incubation time may vary and should be determined empirically by the user. It is recommended that the reaction be stopped when the "Positive Control" wells are lower than ~1.0 absorbance at 450 nm (preferably ~0.6).

18. Add 100 µl of Stop Solution to every well. The blue colored solution will turn yellow.
19. Read the absorbance at λ=450 nm using an UV/Vis spectrophotometer microplate reader.

Example Results

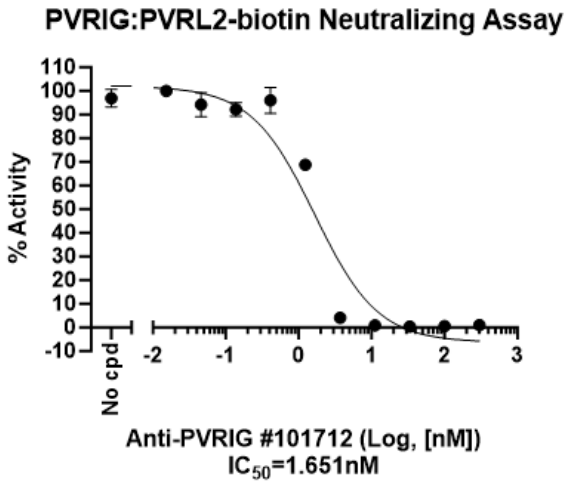

Figure 2. Inhibition of CD112R (PVRIG):CD112 (PVRL2) binding by anti-CD112R (PVRIG) neutralizing antibody.
CD112R:CD112 binding was evaluated in the presence of increasing concentrations of Anti- PVRIG Neutralizing Antibody (BPS Bioscience #101712). Results are expressed as percent activity, in which the binding activity 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

Zhu Y., *et al.*, 2016 *J. Exp. Med.* 213(2):167-76.
Torphy R., *et al.,* 2017 *Int. J. Mol. Sci.*, 18 (12): E2642.
Zeng T., *et al.*, 2021 *Journal of Experimental & Clinical Cancer Research* 40 : 285.

Related Products

Products	*Catalog #*	*Size*
Anti-PVRIG Neutralizing Antibody	101712	50 µg/100 µg
CD112, His-tag (Human) HiP™	71197	100 µg
CD112, His-tag, Biotin-labeled (Human) HiP™	71234	50 µg
CD112, Fc-Fusion, Avi-Tag, (Human)	11079	100 µg
CD112, Fc-Fusion, Avi-Tag, Biotin-labeled (Human)	72231	25 µg/50 µg
CD112R (PVRIG), Fc-Fusion, Avi-Tag (Human) HiP™	79116	100 µg
CD112R (PVRIG), Fc-Fusion, Avi-Tag, Biotin (Human) HiP™	79270	25 µg/50 µg
TIGIT:CD112 Homogeneous Assay Kit	72030	384 reactions
CD226:CD112 Homogeneous Assay Kit	72051	384 reactions

Version 120523