Description

The IL-2: IL-2 Receptor Alpha (IL-2RA) Inhibitor Screening Colorimetric Assay Kit is designed for the screening and profiling of inhibitors or neutralizing antibodies blocking the interaction between human IL-2 and its receptor IL-2RA. The kit is provided in a convenient 96-well format, with Biotinylated-IL-2, purified IL-2RA protein, Streptavidin-HRP, and assay buffers for 100 reactions.

The assay requires only a few steps. First, IL-2RA is coated on a 96-well plate overnight. After blocking, the receptor is pre-incubated with the test inhibitor or neutralizing antibody. Upon subsequent incubation with Biotin-IL-2, 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.

Background

Interleukin-2 (IL-2) is a cytokine secreted by activated T lymphocytes to stimulate the proliferation of both B and T cells. IL-2 is involved in the extreme inflammation response termed “cytokine storm” observed following viral infection in susceptible patients or following adaptive cell therapy. Thus, it is considered a therapeutic target for the treatment of severe COVID-19.

IL-2 acts by binding to a membrane heterotrimeric receptor consisting of IL-2 receptor alpha (IL2RA, also known as CD25) and beta (IL2RB) chains, which together with the common gamma chain (IL2RG) form a high-affinity receptor, whereas IL-2RA homodimers form a low-affinity receptor. Polymorphisms in the genes coding for IL2RA and IL2RB may be associated with risk of lung cancer.

Applications

Screen inhibitors of **IL-2** binding to **IL-2RA.**

Supplied Materials

Catalog #	Name	Amount	Storage
101203	IL-2RA, Avi-His Tag*	2 x 10 µg	-80°C
101381	IL-2, Fc-Avi-Tag, Biotin-Labeled*	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	10 µl	+4°C
79651	Colorimetric HRP substrate	10 ml	+4°C
79964	Transparent 96-well microplate	1	Room Temp

**The initial concentration of both IL-2RA and IL-2 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 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

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

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should contain a "Blank" condition
- We recommend preincubating 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.

Day 1-Coating the plate with IL-2RA protein:

1) Thaw **IL-2RA protein** on ice. Briefly spin the tube to recover its full contents. Aliquot into single use aliquots depending on how many times the plate will be used and immediately store the unused aliquots at -80°C.

   *Note: **IL-2RA protein** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-used the diluted protein.*

2) Dilute **IL-2RA protein** to 4 µg/ml in PBS.

3) Add 50 µl of diluted **IL-2RA protein** solution to each well and incubate at 4°C overnight.

4) Add 50 µl of PBS to "no coat" wells.

Day 2:

5) Prepare **1x Immuno Buffer** by diluting **3x Immuno Buffer** in distilled water: one-part 3x Immuno Buffer in two parts distilled water.

6) After the overnight coating, discard the solution by flipping the plate over waste container or sink, then tap the plate onto paper towels. Wash the plate three times with 100 µl of **1x Immuno Buffer 1** per well. Tap the plate onto clean paper towels to remove the liquid.

7) Block by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature with slow shaking. Remove the blocking solution and tap to dry.

****Note there are two methods for steps 8-12 depending on your inhibitor****

If testing an anti-IL-2RA (CD25) antibody as inhibitor, follow Steps 8-12 below:

8) Prepare dilutions of neutralizing anti-IL-2RA antibody in **Blocking Buffer 2** to desired concentration (it is recommended to use serial dilutions). Prepare enough for 50 µl per well.

9) Add 50 µl of the diluted antibody to the "Test Inhibitor" wells. To wells designated "Blank" and "Positive Control", add 50 µl of **Blocking Buffer 2.** Incubate the plate for 30 minutes (up to 1 hour) at room temperature with slow rotation.

10) Meanwhile, thaw the **IL-2-Biotin** on ice, and dilute it to 2 µg/ml in **Blocking Buffer 2.** Prepare only the amount required for the assay (50 µl /well); Aliquot into single use aliquots depending on how many times the plate will be used and immediately store the unused aliquots at -80°C.

    *Note: **Biotin-IL-2** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-used the diluted protein.*

11) After the antibody incubation, add 50 µl of diluted **IL-2-Biotin** to the wells labeled "Test Inhibitor" and "Positive Control". Add 50 µl **Blocking Buffer 2** to the wells labeled "Blank". At this step, there should be a total of 100 µl in each well.

12) Incubate the plate at room temperature for another 1 hour with slow rotation.

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

After 1 hour, discard the solution and wash the plate three times with **1x Immuno Buffer 1**. Proceed to step 13.

If testing a small molecule inhibitor, follow steps 8-12 below:

8) Prepare the Test Inhibitor.

    a) If the Test Inhibitor is dissolved in DMSO, prepare a solution at a concentration 100-fold higher than the final desired concentration. Further dilute 10-fold in distilled water so it is 10-fold higher than the desired final concentration.

    For example, to test a compound at 10 µM, prepare the inhibitor in DMSO at 1 mM. Then make a 10-fold dilution in distilled water to obtain a 100 µM solution in 10% DMSO.

    b) If the compound is soluble in water, prepare a solution of the compound in distilled water that is 10-fold higher than the final desired concentration.

9) Add 5 µl of Test Inhibitor to each well labeled "Test Inhibitor". To the "Positive Control" and "Blank" wells, add 5 µl of the diluent solution without inhibitor (e.g. 10% DMSO solution in distilled water or distilled water) so that all wells contain the same amount of DMSO.

Caution! – It is highly recommended that the final DMSO concentration does not exceed 1%. Organic solvents other than DMSO have not been validated in this assay, so use of these solvents must be optimized by the user.

10) Thaw the **Biotin-IL-2** on ice, and dilute it in **Blocking Buffer 2** at 2 µg/ml. Prepare only the amount required for the assay; aliquot into single use aliquots depending on how many times the plate will be used and store the remaining **Biotin-IL-2** undiluted at -80°C.

    Note: Biotin-IL-2 is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-used the diluted protein.

11) Add 20 µl of **Blocking Buffer 2** to the wells labeled "Blank". Add 20 µl of diluted **Biotin-IL-2** to the wells labeled "Test Inhibitor" and "Positive Control". Incubate the plate at room temperature for 1 hour with slow rotation.

	Blank	**Positive Control**	**Test Inhibitor**
Blocking Buffer 2	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent solution (no inhibitor)	5 µl	5 µl	-
IL-2-Biotin (2 µg/ml)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

12) After 1 hour, discard the solution and wash the plate three times with 100 µl of **1x Immuno Buffer 1**. Tap the plate onto clean paper towels to remove the liquid.

Day 2-Detection:

13) Dilute **Streptavidin-HRP** 1000-fold with the **Blocking Buffer 2,** enough for 50 µl per well.

14) Add 50 µl of the **diluted Streptavidin-HRP** to each well and incubate the plate for 30 minutes at room temperature with slow rotation.

15) After 30 minutes, discard the solution and wash the plate three times.

16) Meanwhile, prepare enough 1M HCl (aqueous-stop solution) for 100 µl per well. *Note: Alternatively, 2N H_2SO_4 or other compatible acidic solutions can be substituted.*

17) Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until blue color is developed in the 'Positive Control' wells. 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' well is lower than ~ 1.0 absorbance at 450 nm (preferably ~ 0.6).

18) Once a blue color has developed in the 'Positive Control' well, add 100 µl of **1M HCl stop solution** prepared above to every well. The blue color should turn yellow.

19) Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader.

Example Results

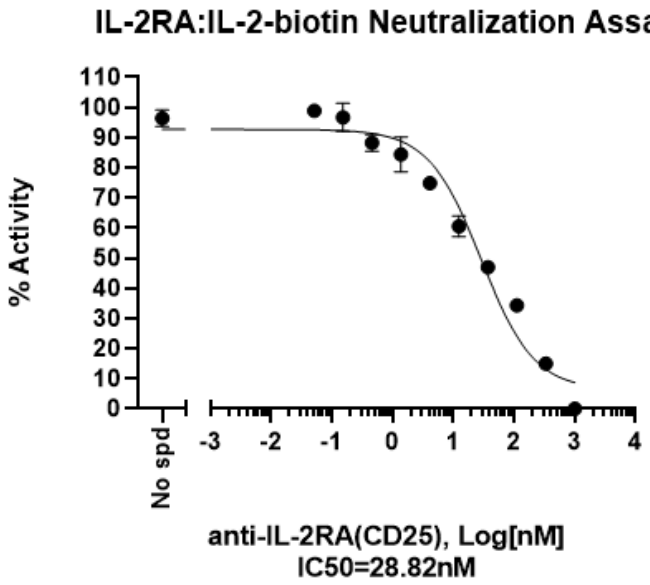

Figure 1: Inhibition of IL-2RA: IL-2 binding by an IL-2RA inhibitor.
Anti-IL-2RA (CD25) antagonist antibody (BPS Bioscience #101593) was evaluated using the IL-2: IL-2RA Inhibitor Screening Colorimetric Assay Kit. The antibody was serially diluted from 1 µM in 3-fold dilutions and tested following the assay kit protocol.
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.

Related Products

Products	*Catalog #*	*Size*
Anti-IL-2RA (CD25) Antagonist Antibody	101593	50 µg/100 µg
IL-2, Fc-Avi-Tag, Biotin labeled	101381	25 µg/100 µg
IL-2RA, Avi-His Tag	101203	100 µg
IL-2 Luciferase Reporter Jurkat Cell Line	60481	2 vials
IL-2 (C145A) Recombinant	100159	100 µg