Description

The Growth and differentiation factor 15 (*GDF15):GFRAL[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of GDF15:GFRAL signaling. This kit comes in a 96-well format, with biotin-labeled GFRAL, purified GDF15, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled GFRAL by streptavidin-HRP. First, GDF15 is coated on a 96-well plate. Next, GDF15 is co-incubated with inhibitor(s) and biotin-conjugated GFRAL on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of a colorimetric HRP substrate. The reaction is quenched, and absorbance is measured on a plate reader.

Background

Growth and differentiation factor 15 (GDF15) is a cytokine, member of the TGF-β superfamily, and binds to the receptor Glial-derived neurotrophic factor-family receptor α-like (GFRAL). GDF15 is not normally expressed in tissues and is thought to be induced at times of stress. GDF15 is considered a biomarker for inflammation and adverse cardiovascular events. Elevations in GDF15 reduce food intake and body mass through binding to GFRAL and the recruitment of the tyrosine kinase RET in the hindbrain. Due to the role of GDF15 in suppressing inflammation and appetite, it is a promising target to treat metabolic diseases including obesity, type 2 diabetes, non-alcoholic fatty liver disease, cardiovascular disease, and cancer cachexia.

Application(s)

Screen inhibitors or neutralizing antibodies of GDF15 binding to GFRAL.

Supplied Materials

Catalog #	Name	Amount	Storage
	GDF15*	20 µg	-80°C
101013	GFRAL, Fc Fusion, Avi-Tag, Biotin-Labeled*	10 µg	-80°C
79742	Streptavidin-HRP	10 µl	+4°C
79311	3x Immuno Buffer	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79651	Colorimetric HRP Substrate	10 ml	+4°C
79964	Transparent 96-well microplate	1	Room Temperature

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

Materials Required but Not Supplied

- PBS (Phosphate buffered saline)
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to one year from date of receipt when the materials are stored as directed.

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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 final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

All samples and controls should be tested in duplicate.

Coating the plate with GDF15:

1. Thaw **GDF15** on ice. Briefly spin the tube to recover the full contents. If the assay plate is going to be used more than once, prepare enough GDF15 for this portion of the assay and aliquot the remaining undiluted protein into single-use aliquots depending on how many times the assay plate will be used. Immediately store unused **GDF15** aliquots at -80°C.

   *Note: **GDF15** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2. Dilute **GDF15** to 4 µg/ml in PBS.

3. Add 50 µl of diluted **GDF15** solution to each well and incubate overnight at 4°C. Leave a couple of wells empty (uncoated), for use with the "Uncoated Control" (see below).

4. The next day, dilute **3x Immuno Buffer** to **1x Immuno Buffer** with water by adding one part of **3x Immuno Buffer** to two parts of distilled water.

5. Remove the **GDF15** solution and wash the plate 3 times with 100 µl of **1x Immuno Buffer**. Tap the plate onto clean paper towels to remove excess liquid.

6. Block by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature.

Step 1:

1. Prepare dilutions of inhibitor or neutralizing antibody in **Blocking Buffer 2** to the desired final concentration (it is recommended to use serial dilutions). Prepare enough for 50 µl per well.

2. Remove the blocking buffer from the plate and tap the plate onto clean paper towels.

3. Add 50 µl of the diluted inhibitor/neutralizing 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 at room temperature with slow shaking.

4. Meanwhile, thaw the **Biotin-GFRAL** on ice, and dilute it to 1.5 ng/µl in **Blocking Buffer 2**. If the assay plate is going to be used more than once, prepare enough **Biotin-GFRAL** for this portion of the assay (50 µl/well) and aliquot the remaining undiluted protein into single-use aliquots depending on how many times the assay plate will be used. Store the unused, undiluted **Biotin-GFRAL** at -80°C.

5. After the inhibitor/neutralizing antibody incubation, add 50 µl of diluted **Biotin-GFRAL** to the wells labeled "Test Inhibitor" and "Positive Control". Add 50 µl of **Blocking Buffer 2** to the wells labeled "Blank". The total volume is 100 µl/well. Incubate the plate at room temperature for another hour with slow agitation/rocking.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Blocking Buffer 2	100 µl	50 µl	-
Test inhibitor/neutralizing antibody	-	-	50 µl
GFRAL-Biotin (1.5 ng/µl)	-	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

6. After 1 hour, discard the solution and wash the plate three times with **1x Immuno Buffer 1.**

7. Dilute **Streptavidin-HRP** 1000-fold with the **Blocking Buffer 2**, enough for 100 µl per well.

8. Add 100 µl of the diluted **Streptavidin-HRP** to each well and incubate the plate for 30 minutes at room temperature with slow rotation.

9. After 30 minutes, discard the solution and wash the plate three times with 100 µl of **1x Immuno Buffer 1**.

10. Prepare **1N HCl** (aqueous solution) for 100 µl/well. Alternatively, 2N H_2SO_4 or other compatible acidic solutions can be substituted.

11. Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until a blue color has 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).

12. Once a blue color has developed in the 'Positive Control' well, add 100 µl of **HCl** stop solution to every well. The blue color should turn yellow. Read the absorbance at 450 nm using UV/Vis spectrophotometer plate reader.

Validation Data:

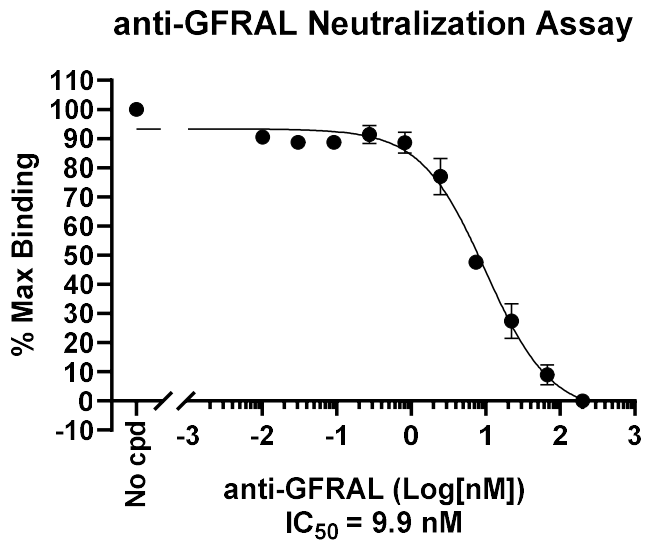

Figure 1. *Inhibition of GDF15:GFRAL interaction by anti-GFRAL neutralizing antibody (BPS Bioscience #101351).*

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-GFRAL Neutralizing Antibody	101351	50 µg
GFRAL, Fc Fusion, Avi-Tag Recombinant	101012	100 µg, 1 mg
GFRAL, Fc Fusion, Avi-Tag, Biotin-Labeled Recombinant	101013	20 µg, 50 µg