

**Description**

The FGR Kinase Assay Kit is designed to measure FGR kinase activity for screening and profiling applications using ADP-Glo™ as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant FGR, substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

**Background**

FGR is a tissue-specific member of the Src tyrosine kinase family of proteins, and it is involved in Fcγ receptor signaling pathways in phagocytosis and mitochondrial oxidative phosphorylation. This protein is overexpressed in several cancer types, such as AML (acute myeloid leukemia), and has also been linked to Alzheimer's disease (AD), Parkinson's disease (PD), pain, diabetes, and schizophrenia. Activation of the FGR/SIRT1 (sirtuin 1)/PGC-1α (peroxisome proliferator-activator receptor γ coactivator 1α) pathway may also be involved in sepsis. Interestingly, it has been found that obesity can lead to FGR activation in macrophages, and contribute to inflammation, making the development of strategies targeting the mitochondrial ROS (reactive oxidative species)-FGR axis clinically relevant in obesity. A deeper understanding of the role of this protein in health and disease will open new therapeutic avenues for several FGR linked disorders.

**Applications**

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

**Supplied Materials**

Catalog #	Name	Amount	Storage
	FGR*	5 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	50 µl	-20°C
40217	PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	50 µl	-20°C
79696	White 96-well plate	1	Room Temperature

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

**Materials Required but Not Supplied**

Name	Ordering Information
ADP-Glo™ Kinase Assay	Promega #V6930
DTT (Dithiothreitol), 1M, optional	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

**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.

**Assay Principle**

The **ADP-Glo™ Kinase Assay (Promega #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear to 1 mM ATP.

**Contraindications**

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

**Assay Protocol**

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs ([bpsbioscience.com](http://bpsbioscience.com)).
- We recommend using TL02-59 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<sub>50</sub> value shown in the validation data below.

1. Thaw **5x Kinase Assay Buffer 1**, **500 μM ATP**, and **PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)**.

*Optional: If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.*

2. Prepare 3 ml of **1x Kinase Assay Buffer 1** by mixing 600 μl of **5x Kinase Assay Buffer 1** with 2,400 μl of distilled water.

*Note: Three (3 ml) of **1x Kinase Assay Buffer 1** is sufficient for 100 reactions.*

3. Prepare a **Master Mix** (12.5 μl/well): N wells x (6 μl of 5x Kinase Assay Buffer 1 + 0.5 μl of 500 μM ATP + 0.5 μl of PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml) + 5.5 μl of distilled water).
4. Add 12.5 μl of Master Mix to every well.
5. Prepare the **Test Inhibitor** (2.5 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 25 μl.

5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Kinase Assay Buffer 1, 10-fold more concentrated than the desired final concentrations.

For the positive and negative controls, use 1x Kinase Assay Buffer 1 (Diluent Solution).

**OR**

5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer 1 to keep the concentration of DMSO constant.

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

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

6. Add 2.5  $\mu$ l of Test Inhibitor to each well labeled "Test Inhibitor".
7. Add 2.5  $\mu$ l of Diluent Solution to the "Positive Control" and "Blank" wells.
8. Add 10  $\mu$ l of 1x Kinase Assay Buffer 1 to the "Blank" wells.
9. Thaw **FGR Kinase** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10  $\mu$ l/well) to 5 ng/ $\mu$ l with **1x Kinase Assay Buffer 1**.
11. Initiate the reaction by adding 10  $\mu$ l of diluted kinase to the wells designated "Positive Control" and "Test Inhibitor".

<b>Component</b>	<b>Blank</b>	<b>Positive Control</b>	<b>Test Inhibitor</b>
Master Mix	12.5 $\mu$ l	12.5 $\mu$ l	12.5 $\mu$ l
Test Inhibitor	-	-	2.5 $\mu$ l
Diluent Solution	2.5 $\mu$ l	2.5 $\mu$ l	-
1x Kinase Assay Buffer 1	10 $\mu$ l	-	-
Diluted FGR (5 ng/ $\mu$ l)	-	10 $\mu$ l	10 $\mu$ l
<b>Total</b>	<b>25 <math>\mu</math>l</b>	<b>25 <math>\mu</math>l</b>	<b>25 <math>\mu</math>l</b>

12. Incubate at 30°C for 45 minutes.
13. Thaw the ADP-Glo™ reagent.
14. At the end of the 45-minute reaction, add 25  $\mu$ l of ADP-Glo™ reagent to each well.
15. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.
16. Thaw the Kinase Detection Reagent.

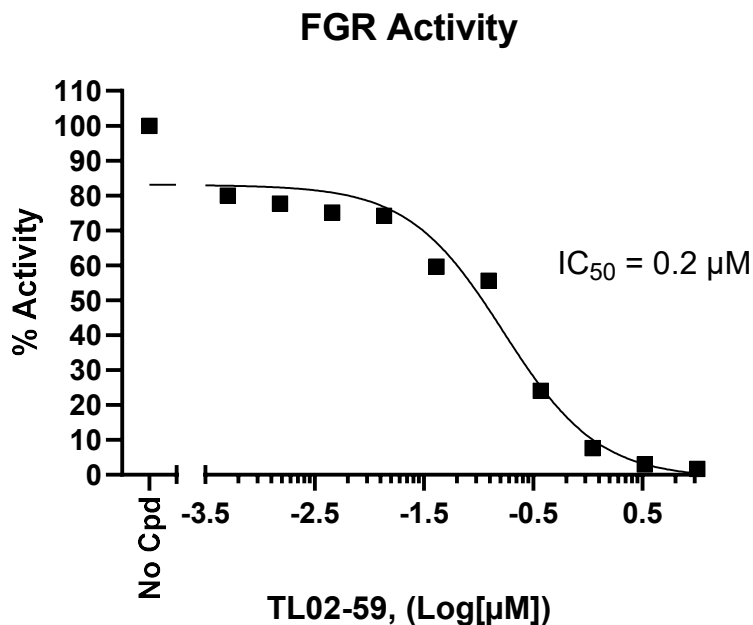
17. Add 50  $\mu\text{l}$  of Kinase Detection reagent to each well.
18. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.
19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.
20. The “Blank” value is subtracted from all other readings.

### Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, 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: 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 1: Inhibition of FGR kinase activity by TL02-59.*

FGR kinase activity was measured in the presence of increasing concentrations of TL02-59 (MedChemExpress #HY-112852). The “Blank” value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

*Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at [support@bpsbioscience.com](mailto:support@bpsbioscience.com).*

**Troubleshooting Guide**

Visit [bpsbioscience.com/assay-kits-faq](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com)

**References**

Liu Y., *et al.*, 2023 *J Transl Med* 21:486.

Acin-Perez R., *et al.*, 2020 *Nat Metab* 2(9): 974-988.

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Sirtuin 1 (193-741), GST-Tag Recombinant	50012	100 µg
SRC Assay Kit	79680	96 reactions
SRC, His-Tag Recombinant	40484	10 µg
SRC, GST-Tag Recombinant	40483	10 µg
LCK, GST-Tag Recombinant	40470	10 µg
CSK, GST-His-Th-Tag Recombinant	40410	10 µg

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