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Data Sheet ***FAK Kinase Assay Kit*** **Catalog # 40722**

Background: FAK (Focal Adhesion Kinase; PTK2) is a protein kinase involving cell adhesion and migration. FAK plays an important role in many cancers including pancreatic tumors. In addition, recent studies showed FAK inhibitor enables an anti-tumor immune response, resulting in tumor destruction in a mouse model.

Description: The *FAK Kinase Assay Kit* is designed to measure FAK kinase activity for screening and profiling applications using Kinase-Glo[®] MAX as a detection reagent. The *FAK Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant FAK enzyme, FAK substrate, ATP and Kinase Buffer 1 for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40420	FAK	10 µg	-80°C	<i>Avoid multiple freeze/thaw cycles!</i>
79334	5x Kinase Buffer 1	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
40217	PTK substrate Poly (Glu:Tyr 4:1) (10 mg/ml)	100 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Kinase-Glo MAX (Promega, #V6071)
Dithiothreitol (DTT, 1 M; optional)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCE: Jiang, H. *et al.*, *Nature Medicine* **22**:851-860 (2016)

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Thaw **5x Kinase Buffer 1**, **ATP** and **PTK substrate Poly (Glu:Tyr 4:1) (10 mg/ml)**.
 (Optional: If desired, add DTT to **5x Kinase Buffer 1** to make a 10 mM concentration; e.g. add 10 µl of 1 M DTT to 1 ml **5x Kinase Buffer 1**)
- 2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase Buffer 1** + 1 µl **ATP (500 µM)** + 1 µl **PTK substrate Poly (Glu:Tyr 4:1) (10 mg/ml)** + 17 µl water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase Buffer 1	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
PTK substrate (10 mg/ml)	1 µl	1 µl	1 µl
Water	17 µl	17 µl	17 µl
Test Inhibitor	-	5 µl	-
Inhibitor Buffer (no inhibitor)	5 µl	-	5 µl
1x Kinase buffer	-	-	20 µl
FAK (4 ng/µl)	20 µl	20 µl	-
Total	50 µl	50 µl	50 µl

- 3) Add 5 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 5 µl of the same solution without inhibitor (Inhibitor buffer).
- 4) Prepare 3 ml of **1x Kinase Buffer 1** by mixing 600 µl of **5x Kinase Buffer 1** with 2400 µl water. 3 ml of **1x Kinase Buffer 1** is sufficient for 100 reactions.
- 5) To the wells designated as "Blank", add 20 µl of **1x Kinase Buffer 1**.
- 6) Thaw **FAK** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **FAK** required for the assay and dilute enzyme to 4 ng/µl with **1x Kinase Buffer 1**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: FAK enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

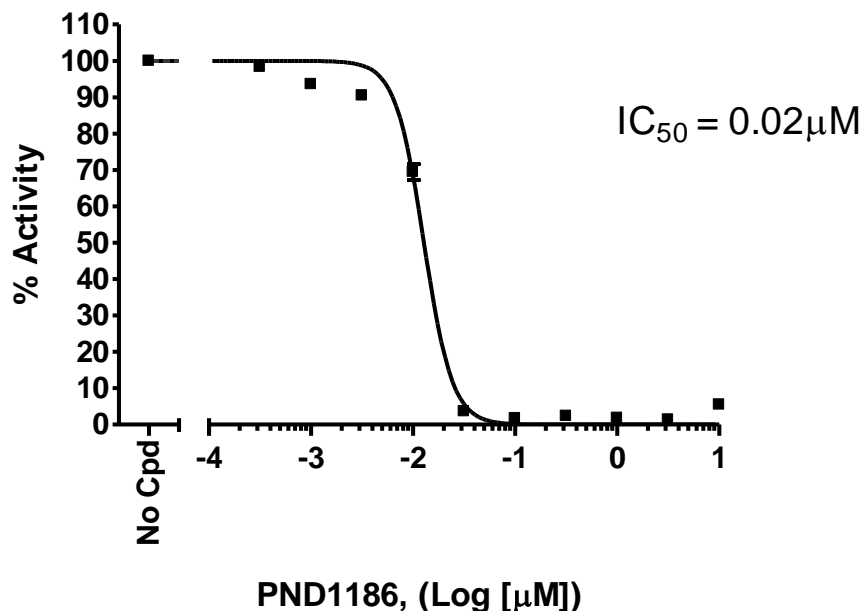
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- 7) Initiate reaction by adding 20 μ l of diluted **FAK** enzyme to the wells designated "Positive Control" and "Test Inhibitor". Incubate at 30°C for 45 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45 minute reaction, add 50 μ l of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
- 10) Measure luminescence using the microplate reader.

Example of Assay Results:**FAK Activity**

Inhibition of FAK enzyme by PND1186, measured using the *FAK kinase assay kit* (Cat. #40722). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
FAK	40420	10 µg
PYK2 (FAK2)	40480	10 µg
EGFR	40187	10 µg
EGFR (L858R)	40189	10 µg
EGFR (T790M)	40188	10 µg
EGFR (T790M, L858R)	40350	10 µg
EGFR (T790M, C797S, L858R)	40351	10 µg
EGFR (mouse)	40195	10 µg

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