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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<sup>®</sup> 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	<b><i>Avoid multiple freeze/thaw cycles!</i></b>
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  $\mu$ l of 1 M DTT to 1 ml **5x Kinase Buffer 1**)
- 2) Prepare the master mixture (25  $\mu$ l per well): N wells x (6  $\mu$ l **5x Kinase Buffer 1** + 1  $\mu$ l **ATP (500  $\mu$ M)** + 1  $\mu$ l **PTK substrate Poly (Glu:Tyr 4:1) (10 mg/ml)** + 17  $\mu$ l water). Add 25  $\mu$ l to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase Buffer 1	6 $\mu$ l	6 $\mu$ l	6 $\mu$ l
ATP (500 $\mu$ M)	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l
PTK substrate (10 mg/ml)	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l
Water	17 $\mu$ l	17 $\mu$ l	17 $\mu$ l
Test Inhibitor	-	5 $\mu$ l	-
Inhibitor Buffer (no inhibitor)	5 $\mu$ l	-	5 $\mu$ l
1x Kinase buffer	-	-	20 $\mu$ l
FAK (4 ng/ $\mu$ l)	20 $\mu$ l	20 $\mu$ l	-
<b>Total</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>

- 3) Add 5  $\mu$ l of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 5  $\mu$ l of the same solution without inhibitor (Inhibitor buffer).
- 4) Prepare 3 ml of **1x Kinase Buffer 1** by mixing 600  $\mu$ l of **5x Kinase Buffer 1** with 2400  $\mu$ l water. 3 ml of **1x Kinase Buffer 1** is sufficient for 100 reactions.
- 5) To the wells designated as "Blank", add 20  $\mu$ l 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/ $\mu$ l 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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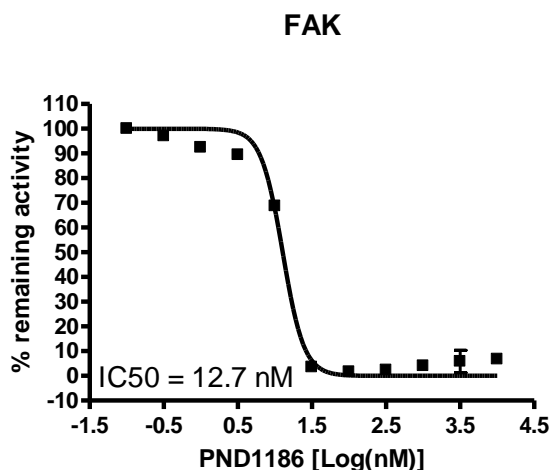
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- 7) Initiate reaction by adding 20  $\mu$ l of diluted **FAK** enzyme to the wells designated "Positive Control" and "Test Inhibitor". Incubate at 30°C for 45 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45 minute reaction, add 50  $\mu$ 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:**



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 info@bpsbioscience.com*

**RELATED PRODUCTS:**

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

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