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Data Sheet

JAK3 (Janus Kinase 3) Assay Kit

Catalog #79521

DESCRIPTION: Janus kinase (JAK) is a family of intracellular, nonreceptor tyrosine kinases that play an important role in cytokine-mediated signaling. This family includes four protein kinases; Jak1, Jak2, Jak3 and Tyk2. Various studies have suggested that regulation of Jak activity should be a promising approach to combat numerous diseases such as immune deficiencies and cancers. The *JAK3 (Janus Kinase 3) Assay Kit* is designed to measure JAK3 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *JAK3 (Janus Kinase 3) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant JAK3 enzyme, JAK3 substrate peptide (Poly(Glu;Tyr, 4:1)), ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40452	JAK3	1 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay 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:
Alicea, N.L. *et al*., *Current Drug Targets* **12(4):**546-555 (2011)

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP** and **PTK Substrate Poly (Glu;Tyr, 4:1) (10 mg/ml)**.
(Optional: If desired, add DTT to **5x Kinase assay buffer 1** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer 1**)

2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer 1** + 1 µl **ATP (500 µM**) + 1 µl **PTK Substrate Poly (Glu;Tyr, 4:1) (10 mg/ml)** + 17 µl distilled water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay 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 1	–	–	20 µl
JAK3 (0.25 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 assay buffer 1** by mixing 600 µl of **5x Kinase assay buffer 1** with 2400 µl water. 3 ml of **1x Kinase assay buffer 1** is sufficient for 100 reactions.

5) To the wells designated as "Blank", add 20 µl of **1x Kinase assay buffer**.

6) Thaw **JAK3** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **JAK3** required for the assay and dilute enzyme to ~0.25 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **JAK3** enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Initiate reaction by adding 20 µl of diluted **JAK3** enzyme to the wells designated “Positive Control” and "Test Inhibitor ". Incubate at 30˚C for 45 minutes.

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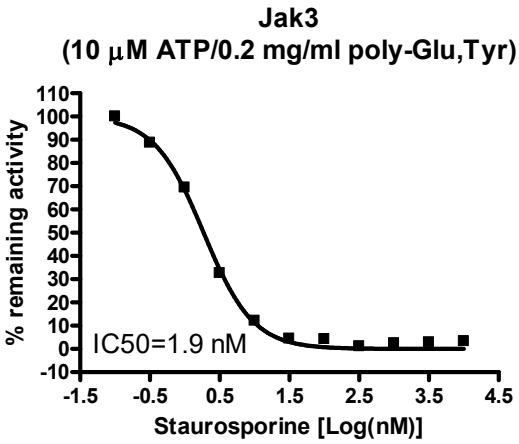
8) Thaw Kinase-Glo Max reagent.

9) After the 45 minute reaction, add 50 µl 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 JAK3 enzyme by Staurosporine, measured using the *JAK3 (Janus Kinase 3) assay kit* (Cat. #79521). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
Jak1, GST-tag	40449	10 µg
Jak2 (JH1 domain), His-tag	40450	10 µg
Jak2 (JH1, JH2 domain), His-GST-tags	40451	10 µg
JAK2-His-Avi-Tag, Biotin-Labeled	79074	10 µg
Jak2 (V617F), GST/Avi-Tag, Biotin-Labeled	79115	10 µg
Jak3, His-tag	40452	10 µg
Tyk2, His-tag	40285	10 µg
ISRE Reporter Kit (JAK/STAT Pathway)	60613	500 rxns.
ISRE Reporter – HEK293 Cell Line	60510	2 vials
Transfection Collection™ : ISRE Transient Pack JAK/STAT Signaling Pathway	79264	500 rxns.
JAK1 (Janus Kinase 1) Assay Kit	79518	96 rxns.
JAK2 (Janus Kinase 2) Assay Kit	79520	96 rxns.
TYK2 (Tyrosine Kinase 2) Assay Kit	79521	96 rxns.

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