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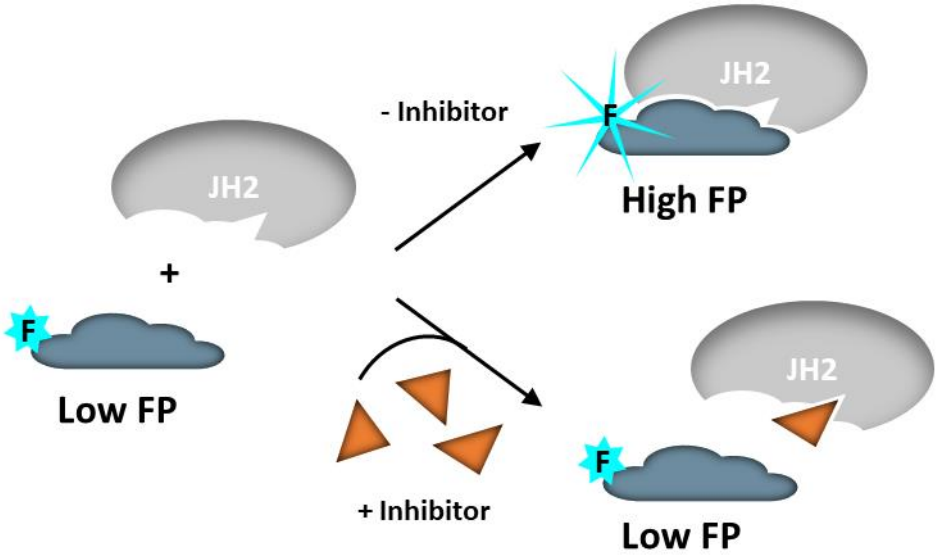
Data sheet
JAK2 JH2 Pseudokinase Domain Inhibitor Screening Assay Kit
Catalog #78111
Size: 384 reactions

BACKGROUND: Janus kinases (JAKs) are a family of intracellular nonreceptor tyrosine kinases including JAK1, JAK2, JAK3 and Tyk2, that has been recognized as an important modulator in inflammatory processes. JAKs contain a catalytically inactive pseudokinase regulatory domain (JH2) as well as an active kinase domain (JH1). Selective inhibition of one specific JAK is a challenging task since the enzymes share high homology in the active site of JH1. Recent reports demonstrate that the pseudokinase domain (JH2) could provide an ideal allosteric site for selective inhibitor development.

DESCRIPTION: The *JAK2 JH2 Pseudokinase Domain Inhibitor Screening Assay Kit* is designed for screening and profiling small molecules that displace the fluorescently labeled probe (JH2 probe 1) from the JH2 domain of JAK2. This kit comes in a convenient 384-well format, with enough recombinant human JAK2 JH2 and fluorescently labeled ATP binding site tracer (JH2 probe 1) for 384 reactions. The assay is based on the competition of the test compound with the JH2 probe for binding to purified JAK2 JH2. Using this kit, only one simple step on a microplate is required for screening. The JH2 probe 1 is incubated with a sample containing JAK2 JH2 to produce a change in fluorescent polarization. The FP signal is measured using a fluorescent microplate reader *capable of measuring fluorescence polarization.*



COMPONENTS:

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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Catalog #	Component	Amount	Storage	
79074	JAK2 (JH2 Domain)	60 µg	-80°C	Avoid multiple freeze/thaw cycles!
78103	JH2 Probe 1, 10 µM *(Protect from light)*	10 µl	-80°C	
78106	JH2 Binding Buffer	25 ml	-20°C	
79961	384-well black microplate	1	Room Temp	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Adjustable micropipettor and sterile tips
Microplate reader capable of reading fluorescence polarization

APPLICATIONS: This kit is useful for screening for small molecules displacing the JH2 probe 1 from the ATP binding site of JAK2 JH2 domain.

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

CONTRAINDICATIONS: Keep final DMSO concentration at or below 1%.

REFERENCES:
Wrobleski, S. T., *et al. J. Med. Chem.* 2019, **62(20)**: 8973-8995.

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

1) Thaw **JH2 Binding Buffer**. JH2 Binding Buffer can be stored at 4˚C for a few days. For long term storage, it is recommended to aliquot and store at -20˚C.
2) Thaw **JAK2 JH2** on ice. Upon first thaw, briefly spin tube containing **JAK2 JH2** to recover full content of the tube. Aliquot **JAK2 JH2** into single use aliquots. Store remaining **JAK2 JH2** in aliquots at -80˚C immediately. Note: **JAK2 JH2** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
3) Dilute test inhibitor into distilled water at 10X testing concentration. (*e.g.*, To test at 100 nM, prepare a 10 µM solution of the test compound in DMSO. Mix 10 µl of the 10 µM solution and 90 µl of distilled water to create a 1 µM testing test compound in 10% DMSO (aqueous) The final concentration of DMSO in the assay must be no more than 1%. For the controls ("Blank", "Reference" and "Positive Control"), prepare 10% DMSO (aqueous) solution (*i.e.*, Inhibitor buffer) by mixing 10 µl DMSO and 90 µl distilled water.
4) Add 45 µl JH2 Binding Buffer to the wells labeled "Blank" (no Probe/no JH2). Add 40 µl JH2 Binding Buffer to the wells labeled "Reference" (with Probe/no JH2).
5) Dilute **JAK2 JH2** in **JH2 Binding Buffer** to ~ 3.5 ng/µl (approximately 90 nM) and add 40 µl to the wells labeled "Positive Control" and "Test Inhibitor". Prepare only the amount required for the assay; discard any remaining diluted JAK2 JH2.

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6) Add 5 µl of the testing inhibitor and Inhibitor buffer prepared in step (3) to the corresponding wells.

	Blank	Reference	Positive Control	Test Inhibitor
JH2 Binding Buffer	45 µl	40 µl	-	-
JAK2 JH2 (~3.5 ng/µl)	-	-	40 µl	40 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	-
Test Inhibitor in 10% DMSO (aqueous)	-	-	-	5 µl
JH2 Probe 1 (30 nM)	-	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 l**

7) Incubate the plate for 10 minutes at room temperature (option: In order to make it sure that all components are mixed well, the plate can be centrifuged at ~ 1,000 rpm for 30 seconds.)

8) During the 10-minute incubation, thaw the **JH2 Probe 1 (10 µM)**.

9) Prepare 30 nM **JH2 Probe 1** solution by diluting **JH2 Probe 1 (10 µM)** in **JH2 Binding Buffer** (*e.g.*, Add 9 µl of **JH2 Probe 1 (10 µM)** to 2,991 µl of **JH2 Binding Buffer**, which is more than enough for 400 reactions)

10) Initiate the binding reaction by adding 5 µl of diluted JH2 Probe 1 to all wells except the wells labeled “Blank”.

11) Incubate the plate for 1 hour at room temperature.

12) Read fluorescent polarization of the sample in a microplate reader capable of excitation at 470 ± 5 nm and detection of emitted light at 530 ± 10 nm in both parallel and perpendicular channels. The “Blank” value is subtracted from all other values.

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CALCULATING RESULTS:

Definition of Fluorescence Polarization:

$$P = \frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}$$

Where $I_{\parallel}$ = Intensity with polarizers parallel and $I_{\perp}$= Intensity with polarizers perpendicular. Most instruments display fluorescence polarization in units of mP.

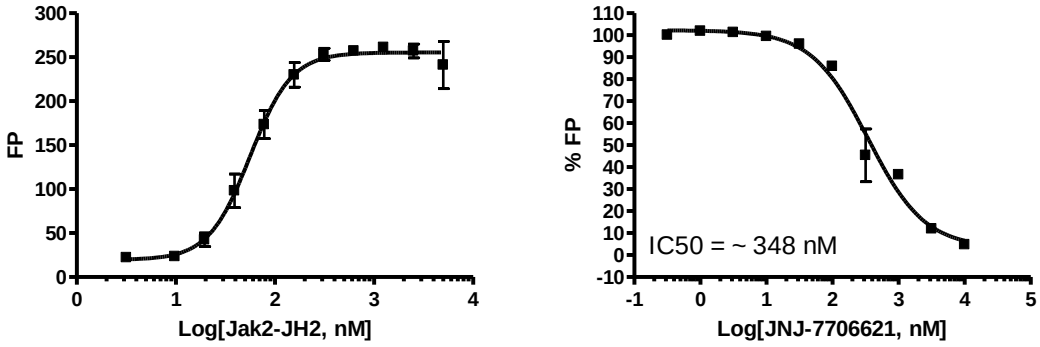
$$mP = \left(\frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}\right) x\ 1000$$

The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000 \qquad \textbf{OR} \qquad mP = \left(\frac{G(I_{II}) - I_{\perp}}{G(I_{II}) + I_{\perp}}\right) x\ 1000$$

The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

Example of assay results:



Binding of JH2 Probe 1 to JAK2 JH2 domain (left) – Various concentrations of JAK2 JH2 domain were incubated with 3 nM JH2 Probe 1 for 1 hour at room temperature followed by measuring fluorescence polarization.

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Inhibition of JH2 Probe 1 binding to JAK2 JH2 domain by JNJ-7706621 (right) – JNJ-7706621 inhibition was measured using the *JAK2 JH2 Pseudokinase Domain Inhibitor Screening Assay Kit*. Fluorescence Polarization was measured using a Tecan fluorescent microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

RELATED PRODUCTS:

Product Name	Catalog#	Size
JAK2 (JH2 domain), His-Avi-Tag, Biotin-Labeled	79074	10 µg
JAK2 (JH1 domain), His-tag	40450	20 µg
JAK2 (JH1, JH2 domain), His-GST-tags	40451	20 µg
JAK2 (V617F) (JH1, JH2 Domain), GST-Tag	79382	20 µg
JAK2 (V617F) (JH2 Domain), His-Avi-Tag, Biotin-Labeled	79075	10 µg
JAK1 (Janus Kinase 1) Assay Kit	79518	96 reactions
JAK2 (Janus Kinase 2) Assay Kit	79520	96 reactions