



# SZABO SCANDIC

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- Expressversand

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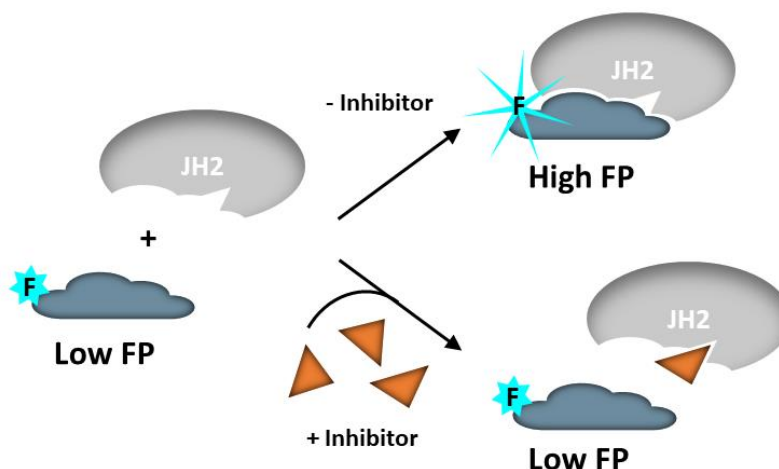
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## Description

The JAK1 (JH2 Pseudokinase Domain) Inhibitor Screening Assay Kit is a fluorescence polarization-based assay designed for screening and profiling small molecules that displace the fluorescently labeled probe (JH2 probe 1) from the JH2 pseudokinase domain of JAK1. This kit comes in a convenient 384-well format, with enough recombinant human JAK1 (JH2 Pseudokinase Domain), buffer, and fluorescently labeled JH2 probe 1 for 384 reactions.

This assay requires a fluorescent microplate reader *capable of measuring fluorescence polarization (FP)* to read the FP signal. For more information on the principles of FP, visit [fp\\_assays.pdf](https://www.bpsbioscience.com/fp_assays.pdf) ([bpsbioscience.com](https://www.bpsbioscience.com)).



**Figure 1: Illustration of the assay principle.** The assay is based on the competition between the test compound and the JH2 probe for the purified JAK1 (JH2 Pseudokinase Domain). JH2 probe 1 is incubated with JAK1 (JH2 Pseudokinase Domain) in the presence or absence of inhibitor. When the JH2 probe 1 is bound to the JH2 pseudokinase domain it has high FP due to its restricted movement. A competitive inhibitor prevents the probe from binding to the JH2 pseudokinase domain, therefore most of the probe is in free form and has low FP. Compared to a control without inhibitor, FP decreases proportionally to the inhibitor concentration.

## Background

Janus kinases (JAKs) are a family of intracellular non-receptor tyrosine kinases, including JAK1, JAK2, JAK3 and TYK2, important in the modulation of inflammatory processes. JAKs contain a catalytically inactive pseudokinase regulatory domain (JH2), that acts as a negative regulator, as well as an active kinase domain (JH1). Most of the mutations in JAK proteins that link to hematological and immune-related diseases occur in the JH2 domain, resulting in increased JAK2 activity or decreased cytokine-induced signaling. Most inhibitors developed so far target the JH1 domain and seem unable to fully treat the disease, while generating significant side effects by suppressing normal cytokine signaling. Recent reports demonstrate that the pseudokinase domain of JH2 could provide an ideal site for selective inhibitor development and support the treatment of diseases like myeloproliferative neoplasms (MNs), with minimal side effects.

## Applications

Screening & profiling of small molecules able to displace the JH2 probe 1 from the ATP binding site of JAK1 (JH2 Pseudokinase Domain).

**Supplied Materials**

Catalog #	Name	Amount	Storage
	JAK1 (JH2 Pseudokinase Domain)*	14 µg	-80°C
78103	JH2 Probe 1, 10 µM ( <i>Protect from light</i> )	10 µl	-80°C
78106	JH2 Binding Buffer	25 ml	-20°C
79961	384-well microplate, black	1 plate	Room Temp

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

**Materials Required but Not Supplied**

- Microplate reader capable of reading fluorescence polarization
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

**Storage Conditions**

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed. **Avoid multiple freeze/thaw cycles!**

**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.

**Contraindications**

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

**Assay Protocol**

- All samples and controls should be performed in duplicate.
  - The assay should include “Blank”, “Reference”, “Positive Control” and “Test Inhibitor” conditions.
  - If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.
- 1) Thaw **JH2 Binding Buffer**. JH2 Binding Buffer can be stored at 4°C up to a week. For long term storage, it is recommended to aliquot and store at -20°C.
  - 2) Thaw **JAK1 (JH2 Pseudokinase Domain)** on ice. Briefly spin the tube containing JAK1 (JH2 Pseudokinase Domain) to recover the full content of the tube.

- 3) Calculate the amount of protein required for the assay (10 µl/well) and dilute to 3.5 ng/µl (approximately 100 nM) with JH2 Binding Buffer. Store the remaining undiluted JAK1 (JH2 Pseudokinase Domain) at -80°C in single-use aliquots.

*Note: JAK1 (JH2 Pseudokinase Domain) is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use the diluted protein.*

- 4) Add 10 µl of diluted JAK1 (JH2 Pseudokinase Domain) to the “Positive Control” and “Test Inhibitor” wells.
- 5) Add 20 µl of JH2 Binding Buffer to the “Blank” (no probe/no protein) wells.
- 6) Add 10 µl of JH2 Binding Buffer to the “Reference” (with probe/no protein) wells.
- 7) Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

7.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 5-fold more concentrated than the desired final concentrations using JH2 Binding Buffer. JH2 Binding Buffer is the Diluent Solution.

**OR**

7.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO, at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 20-fold in JH2 Binding Buffer to prepare the highest concentration of the 5-fold intermediate serial dilutions. The concentration of DMSO is now 5%.

Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in JH2 Binding Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 5% DMSO in JH2 Binding Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO should not exceed 1%.

- 8) Add 5 µl of the Test Inhibitor solution to each well designated as “Test Inhibitor”.
- 9) Add 5 µl of Diluent Solution to the “Positive Control”, “Reference” and “Blank” wells.
- 10) Incubate at Room Temperature (RT) for 10 minutes.

*Note: We strongly recommend pre-incubation of JAK1 (JH2 Pseudokinase Domain) with the inhibitor before adding the probe.*

- 11) Thaw **JH2 Probe 1** (10 µM).

- 12) Prepare 7.5 nM JH2 Probe 1 Solution by diluting JH2 Probe 1 (10  $\mu$ M) in JH2 Binding Buffer. You will need 10  $\mu$ l/well. For example, add 3  $\mu$ l of JH2 Probe 1 (10  $\mu$ M) to 3,997  $\mu$ l of JH2 Binding Buffer. Dilute only enough probe required for the assay. Store remaining JH2 Probe 1 (10  $\mu$ M) at -80°C in single-use aliquots.
- 13) Initiate the reaction by adding 10  $\mu$ l of diluted JH2 Probe 1 Solution to all wells, except the ones designated as “Blank”.
- 14) Incubate at RT for 1 hour protected from light.

	Blank	Reference	Positive Control	Test Inhibitor
Diluted JAK1-JH2 (3.5 ng/ $\mu$ l)	-	-	10 $\mu$ l	10 $\mu$ l
Test Inhibitor	-	-	-	5 $\mu$ l
Diluent Solution	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l	-
JH2 Binding Buffer	20 $\mu$ l	10 $\mu$ l	-	-
Incubate 10 minutes at Room Temperature				
JH2 Probe 1 Solution (7.5 nM)	-	10 $\mu$ 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>	<b>25 <math>\mu</math>l</b>

- 15) Read fluorescent polarization in a microplate reader capable of excitation at  $470 \pm 5$  nm and detection of emitted light at  $530 \pm 10$  nm in both parallel and perpendicular channels.
- 16) Subtract average “Blank” value from all other values.

#### CALCULATING RESULTS:

The user may ignore the G-factor when all experiments are performed using the same instrument since it is instrument-dependent.

If desired, the G-factor should be set before measurements are performed. It needs to be determined by the investigator when not clearly indicated by the manufacturer. The instrument manual will contain information about how to establish the **G-factor**. For example, BPS Bioscience’s scientists use a Tecan fluorescent plate reader which has a G value set to 22 mP.

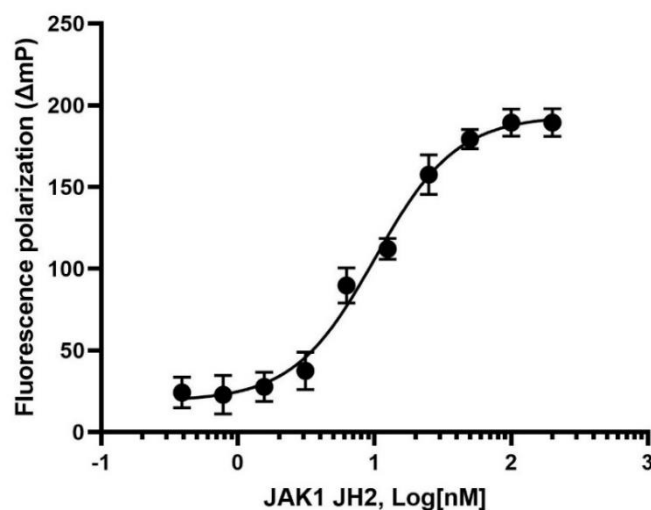
Instruments provide measurement in milli-Polarization = mP.

Calculate  $\Delta$ mP for all sample:

$$\Delta\text{mP} = (\text{mP value of the sample}) - (\text{mP value of the Reference control})$$

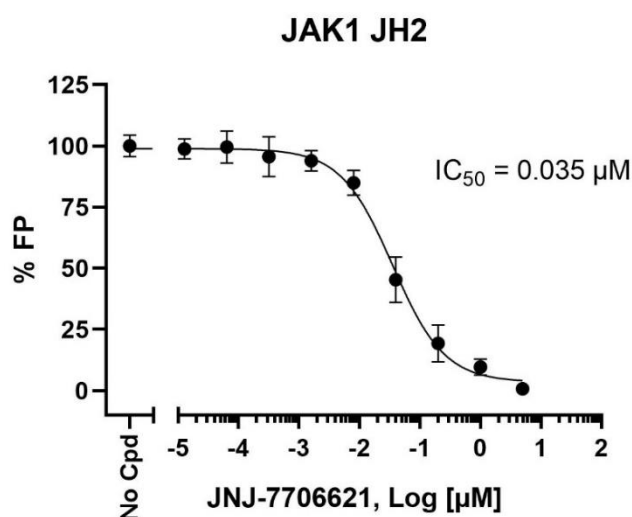
Where mP refers to milli-Polarization values provided by the instrument and Reference control is the mP value obtained in the condition containing only the fluorescent probe (a condition in which the probe is in free state).

## Example Results



*Figure 2: Binding of JH2 Probe 1 to JAK1 (JH2 Pseudokinase Domain).*

Increasing concentrations of JAK1 (JH2 Pseudokinase Domain) were incubated with 3 nM JH2 Probe 1 for 1 hour at room temperature followed by fluorescence polarization measures. Fluorescence Polarization (mP values) increase with the amount of JH2 Probe 1 bound to JAK1 (JH2 Pseudokinase Domain).



*Figure 3: Inhibition of JH2 Probe 1 binding to JAK1 (JH2 Pseudokinase Domain) by JNJ-7706621.*

Inhibition of the probe binding to JAK1 (JH2 Pseudokinase Domain) was measured in the presence of increasing concentrations of JNJ-7706621 (Cayman Chemical #18494). Fluorescence Polarization was measured using a Tecan fluorescent microplate reader. Results are expressed in percent of FP control, where FP in the absence of inhibitor is set to 100%.

*Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

**Troubleshooting Guide**

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

**References**

1. Newton, A. S. *et al.*, 2017 *ACS Med Chem Lett* 8 (6): 614-617.
2. Wroblewski, S. T., *et al.*, 2019 *J Med Chem* 62 (20): 8973-8995.

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
JAK1 (Janus Kinase 1) Assay Kit	79518	96 reactions
JAK2 (Janus Kinase 2) Assay kit	79520	96 reactions
JAK3 (Janus Kinase 3) Assay Kit	79521	96 reactions
TYK (Tyrosine Kinase 2) Assay Kit	79075	96 reactions