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 JAK1 (Janus kinase 1) JH2 Pseudokinase Domain. 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.

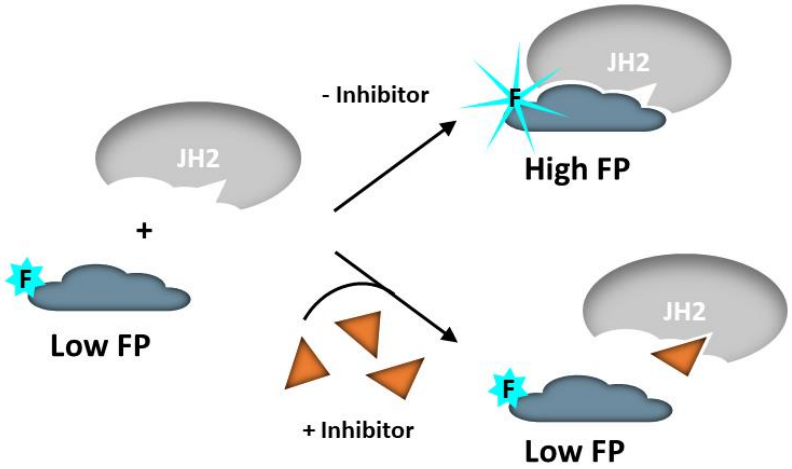

Figure 1: Illustration of the assay principle.

The assay is based on the competition between a 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 an 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 (fluorescence polarization). Compared to a control without inhibitor, FP decreases proportionally to the inhibitor concentration.

This assay requires a fluorescent microplate reader ***capable of measuring fluorescence polarization (FP)*** to read the FP signal. For more information FP technology, visit our Tech Note: FP, assay principles and applications.

Background

Janus kinases (JAKs) are a family of intracellular non-receptor tyrosine kinases, including JAK1, JAK2, JAK3 and TYK2 (tyrosine kinase 2), 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 (MNPs), while generating minimal side effects. The development of such inhibitors will open new avenues in cancer therapy.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
101946	JAK1 (JH2 Pseudokinase Domain), His-Tag*	40 µg	-80°C
78103	10 µM JH2 Probe 1 *(Protect from light)*	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.

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” (no probe/no protein), “Reference Control” (with probe/no protein), “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using JNJ-7706621 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1) Thaw **JH2 Binding Buffer**.

   Note: JH2 Binding Buffer can be stored at 4°C for 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 to recover the full content.

3) Dilute JAK1 (JH2 Pseudokinase Domain) to 2.5 ng/µl with JH2 Binding Buffer (40 µl/well).

4) Add 40 µl of diluted JAK1 (JH2 Pseudokinase Domain) to the "Positive Control" and "Test Inhibitor" wells.

5) Add 45 µl of JH2 Binding Buffer to the "Blank" (no probe/no protein) wells.

6) Add 40 µl of JH2 Binding Buffer to the "Reference Control" (with probe/no protein) wells.

7) Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   7.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations using JH2 Binding Buffer.

   For the positive and negative controls, use JH2 Binding Buffer (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 10-fold in JH2 Binding Buffer to prepare the highest concentration of the 10-fold intermediate serial dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in JH2 Binding Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in JH2 Binding Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

8) Add 5 µl of diluted Test Inhibitor 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.

11) Thaw **10 µM JH2 Probe 1**.

12) Dilute 10 µM JH2 Probe 1 in JH2 Binding Buffer 333-fold (5 µl/well), to get a 30 nM solution. For example, add 9 µl of 10 µM JH2 Probe 1 to 2,991 µl of JH2 Binding Buffer.

    Note: Dilute only enough probe required for the assay. Store remaining 10 µM JH2 Probe 1 at -80°C in single-use aliquots (minimum volume of 5 µl).

13) Initiate the reaction by adding 5 µl of diluted JH2 Probe 1 to all wells, except the ones designated as "Blank".

14) Incubate at RT for 1 hour protected from light.

	Blank	**Reference Control**	**Positive Control**	**Test Inhibitor**
Diluted JAK1-JH2 (2.5 ng/µl)	-	-	40 µl	40 µl
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
JH2 Binding Buffer	45 µl	40 µl	-	-
	Incubate 10 minutes at Room Temperature			
Diluted JH2 Probe 1 Solution (30 nM)	-	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

15) Read fluorescent polarization in a microplate reader capable of excitation at λ=470 ± 5 nm and detection of emitted light at λ=530 ± 10 nm in both parallel and perpendicular channels.

16) The "Blank" value should be subtracted 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 ΔmP for all sample:

ΔmP = (mP value of the sample) – (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

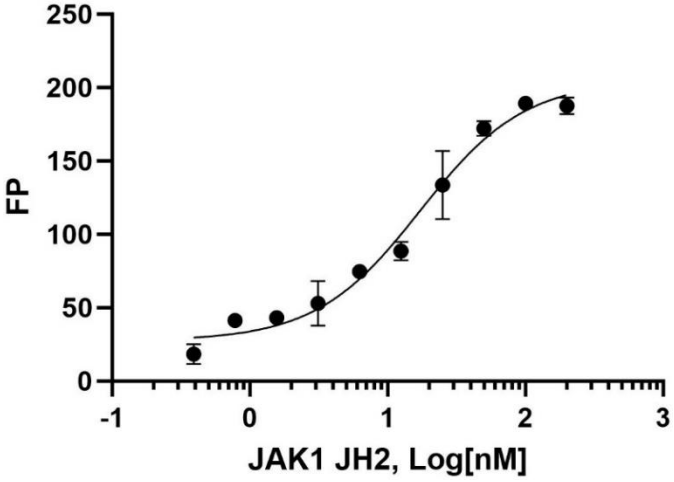

Figure 2: Binding of JH2 Probe 1 to JAK1 (JH2 Pseudokinase Domain).
Increasing concentrations of JAK1 (JH2 Pseudokinase Domain) were incubated with 3 nM JH2 Probe 1 for 1 hour at RT followed by fluorescence polarization measurements. Fluorescence Polarization (mP values) increase with the amount of JH2 Probe 1 bound to JAK1 (JH2 Pseudokinase Domain). Fluorescence Polarization was measured using a Tecan fluorescent microplate reader.

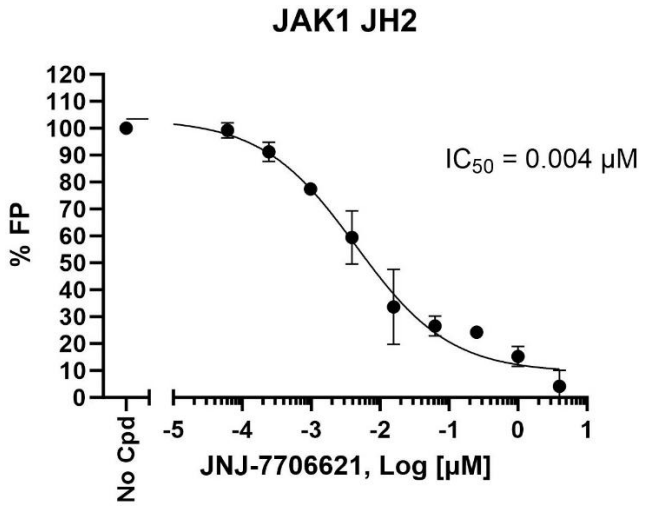

Figure 3: Inhibition of JH2 Probe 1 binding to JAK1 (JH2 Pseudokinase Domain) by JNJ-7706621.
Inhibition of probe binding to JAK1 (JH2 Pseudokinase Domain) was measured in the presence of increasing concentrations of JNJ-7706621 (Cayman Chemical #18494). Fluorescence Polarization was measured using a 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 for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com

References

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

Related Products

Products	*Catalog #*	*Size*
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

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