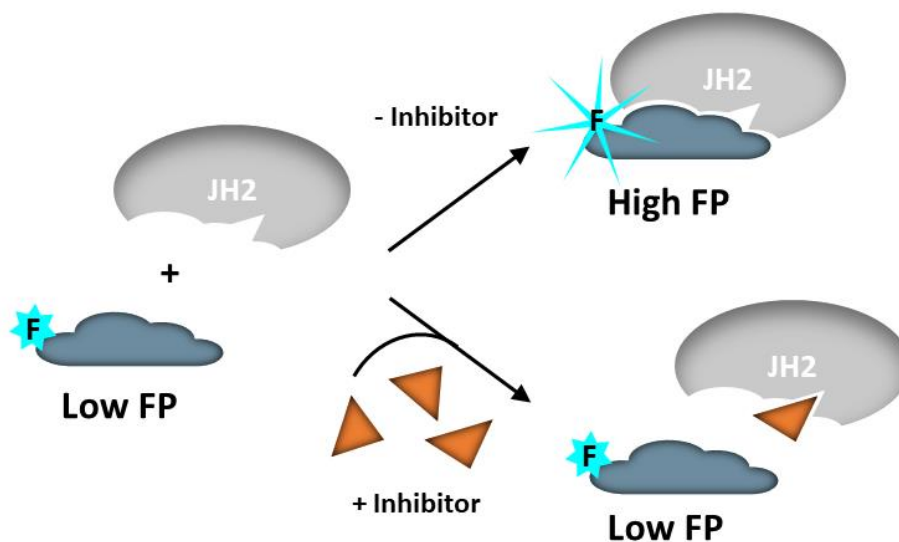


### Description

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. The assay is a fluorescence polarization (FP) assay based on the competition of the test compound with the JH2 probe for binding to purified JAK2 JH2.

JH2 probe 1 is a small molecule probe that binds to the ATP-binding site of JAK2 pseudokinase domain JH2. The probe has been labeled with a fluorescent tracer, so that binding of the probe to much larger JH2 results in noticeable increases in fluorescence polarization compared to the unbound fluorescent probe (depolarized). Competition between a test drug and the probe results in changes in polarization.

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. 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 that can then be measured using a fluorescence microplate reader capable of measuring fluorescence polarization.



### 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 inhibitors

### Applications

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

**Supplied Materials**

| Catalog # | Name   | Amount | Storage   |
|-----------|--|--------|-----------|
| 100124    | JAK2 (JH2 Domain) (V617F, W659A, W777A, F794H)*  | 80 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 black microplate                        | 1      | Room Temp |

\*The concentration of JAK2 is lot-specific and will be indicated on the tube containing the enzyme

**Materials Required but Not Supplied**

- Adjustable micropipettor and sterile tips
- Microplate reader capable of reading fluorescence polarization

**Stability**

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**

Keep final DMSO concentration at or below 1%.

**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 (V617F, W659A, W777A, F794H)** on ice. Upon first thaw, briefly spin tube containing **JAK2 JH2 (V617F, W659A, W777A, F794H)** to recover full content of the tube. Aliquot **JAK2 JH2 (V617F, W659A, W777A, F794H)** into single use aliquots. Store remaining **JAK2 JH2 (V617F, W659A, W777A, F794H)** in aliquots at -80°C immediately. Note: **JAK2 JH2 (V617F, W659A, W777A, F794H)** is 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  $\mu\text{l}$  JH2 Binding Buffer to the wells labeled “Blank” (no Probe/no JH2). Add 40  $\mu\text{l}$  JH2 Binding Buffer to the wells labeled “Reference” (with Probe/no JH2).
- 5) Dilute **JAK2 JH2 (V617F, W659A, W777A, F794H)** in **JH2 Binding Buffer** to  $\sim 5 \text{ ng}/\mu\text{l}$  (approximately 150 nM) and add 40  $\mu\text{l}$  to the wells labeled “Positive Control” and “Test Inhibitor”. Prepare only the amount required for the assay; discard any remaining diluted JAK2 JH2.
- 6) Add 5  $\mu\text{l}$  of the testing inhibitor or Inhibitor buffer prepared in step (3) to the corresponding wells.
- 7) Incubate the plate for 10 minutes at room temperature (option: To ensure that all components are mixed well, the plate can be centrifuged at  $\sim 1,000 \text{ rpm}$  for 30 seconds.)
- 8) During the 10-minute incubation, thaw the **JH2 Probe 1 (10  $\mu\text{M}$ )**.
- 9) Prepare 30 nM **JH2 Probe 1** solution by diluting **JH2 Probe 1 (10  $\mu\text{M}$ )** in **JH2 Binding Buffer** (e.g., Add 9  $\mu\text{l}$  of **JH2 Probe 1 (10  $\mu\text{M}$ )** to 2,991  $\mu\text{l}$  of **JH2 Binding Buffer**, which is more than enough for 400 reactions).
- 10) Initiate the binding reaction by adding 5  $\mu\text{l}$  of diluted JH2 Probe 1 to all wells except the wells labeled “Blank”.
- 11) Incubate the plate for 1 hour at room temperature in dark area.
- 12) Read fluorescent polarization of the sample in a microplate reader capable of excitation at  $470 \pm 5 \text{ nm}$  and detection of emitted light at  $530 \pm 10 \text{ nm}$  in both parallel and perpendicular channels. The “Blank” value is subtracted from all other values.

| Component   | Blank            | Reference        | Positive Control | Test Inhibitor   |
|---|------------------|------------------|------------------|------------------|
| JH2 Binding Buffer  | 45 $\mu\text{l}$ | 40 $\mu\text{l}$ | -                | -                |
| JAK2 JH2 (V617F, W659A, W777A, F794H) ( $\sim 5 \text{ ng}/\mu\text{l}$ ) | -                | -                | 40 $\mu\text{l}$ | 40 $\mu\text{l}$ |
| Inhibitor buffer (no inhibitor)   | 5 $\mu\text{l}$  | 5 $\mu\text{l}$  | 5 $\mu\text{l}$  | -                |
| Test inhibitor in 10% DMSO (aqueous)                                      | -                | -                | -                | 5 $\mu\text{l}$  |
| JH2 Probe 1 (30nM)  | -                | 5 $\mu\text{l}$  | 5 $\mu\text{l}$  | 5 $\mu\text{l}$  |
| Total   | 50 $\mu\text{l}$ | 50 $\mu\text{l}$ | 50 $\mu\text{l}$ | 50 $\mu\text{l}$ |

## Calculating Results

### Definition of Fluorescence Polarization:

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

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

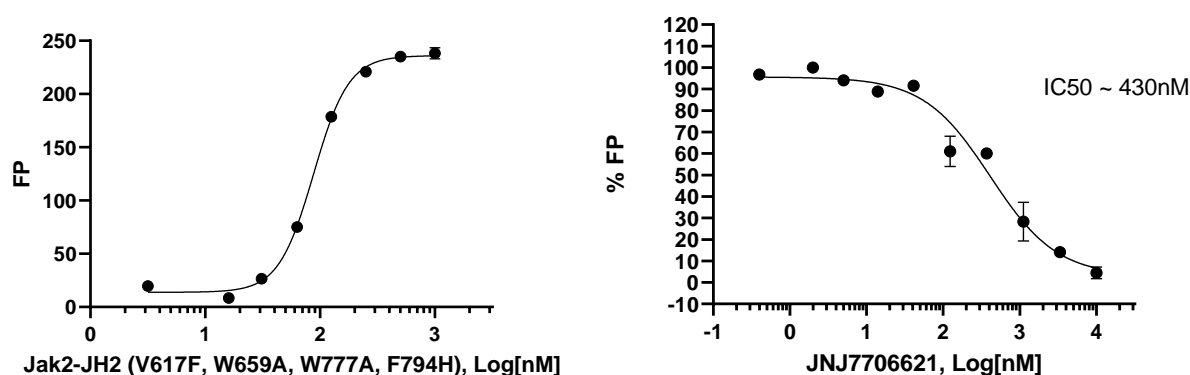
$$mP = \left( \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \right) \times 1000$$

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".

$$mP = \left( \frac{I_{II} - G(I_L)}{I_{II} + G(I_L)} \right) \times 1000 \quad \text{OR} \quad mP = \left( \frac{G(I_{II}) - I_L}{G(I_{II}) + I_L} \right) \times 1000$$

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 Results



#### Binding of JH2 Probe 1 to JAK2 JH2 domain (left)

Increasing concentrations of JAK2 JH2 domain were incubated with 3 nM JH2 Probe 1 for 1 hour at room temperature followed by measurement of fluorescence polarization using a Tecan FP microplate reader.

#### Inhibition of JH2 Probe 1 binding to JAK2 JH2 (V617F, W659A, W777A, F794H) domain by JNJ-7706621 (right)

JNJ-7706621 is a small molecule inhibitor of CDKs, which also binds very effectively to the JAK2 pseudokinase domain JH2. JNJ-7706621 inhibition of JH2 probe 1 binding to JAK2 was measured using the JAK2 JH2 Pseudokinase Domain Inhibitor Screening Assay Kit. Fluorescence Polarization was measured using a Tecan FP reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

#### General considerations

**"Blank" Control:** The "Blank" control is important to determine the background fluorescence in the assay.

#### Troubleshooting Guide

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

## References

1. Wroblewski, S. T., *et al.* *J. Med. Chem.* 2019, **62(20)**: 8973-8995.
2. Hubbard, S. R. "Mechanistic insights into regulation of JAK2 tyrosine kinase." *Frontiers in endocrinology* 2018, **8**: 361.
3. Newton, Ana S., *et al.* "JAK2 JH2 fluorescence polarization assay and crystal structures for complexes with three small molecules." *ACS medicinal chemistry letters* 2017, **8(6)**: 614-617.
4. Yamaoka, Kunihiro, *et al.* "The Janus Kinases (Jaks)." *Genome biology* 2004, **5(12)**: 1-6.

## Related Products

| <i>Products</i>  | <i>Catalog #</i> | <i>Size</i>  |
|--|------------------|--------------|
| 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 |