

LANCE® Ultra

ULight™ -labeled JAK-1 (Tyr1023) Peptide**Product number:** TRF0121-M **Lot Number:** 3407986**Product Format:** TRF0121-D: 0.5 nmole (100µL)

TRF0121-M: 5 nmoles (1mL)

Manufacturing date: 05/12/2025 **Document version:** 1**Product Information****Phosphorylation Motif:** DKEYYTVKD

Synthetic peptide derived from amino acids 1015-1027 of human Janus kinase 1 (JAK-1); phosphorylation site: Tyr1023.

Storage Buffer: 50 mM Tris-HCl (pH 7.4), 0.9% NaCl, 0.1% BSA and 0.05% sodium azide as preservative**Molecular Weight:** 2 726**Stability:** This product is stable for at least **12 months** from the manufacturing date when stored in its original packaging and the recommended storage conditions.**Storage Conditions:** Store at -20°C. Store protected from light. Repeated freezing and thawing should be avoided.**Quality Control**

The QC release specifications are based on spectrophotometric analysis of the labeled protein. We certify that results meet our quality release criteria.

Labeling Ratio: 1 (dye molecule/peptide)**Concentration:** 13.6 µg/mL (10 µM)**Recommended Assay Conditions****JAK3 kinase: ATP titration**

SUGGESTED METHOD:

*(Specific applications might require optimization)*Reagent Preparation

- Prepare 1X Kinase Assay Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl₂, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X JAK3 solution: dilute enzyme to a concentration of 0.5 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X ULight-JAK-1 (Tyr1023) peptide solution: dilute ULight-JAK-1 (Tyr1023) to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 4X ATP solution: dilute ATP to concentrations ranging from 40 nM to 1.2 mM (1/3 serial dilutions) in Kinase Assay Buffer. Keep on ice.
- Prepare 1X Detection Buffer: dilute 0.5 mL of 10X Detection Buffer with 4.5 mL of H₂O.
- Prepare a 4X Stop solution*: dilute EDTA to a concentration of 40 mM in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute Europium-anti-phospho-tyrosine antibody (PT66) to a concentration of 8 nM in 1X Detection Buffer.

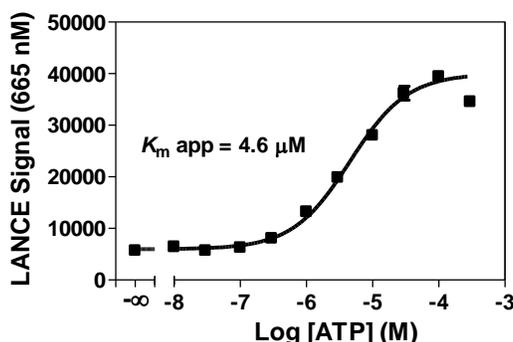
Protocol

- Pipet 5 μ L of 2X JAK3 solution into a 384-well white OptiPlate-384 (0.25 nM final concentration).
- Add 2.5 μ L of 4X *ULight*- JAK-1 (Tyr1023) peptide solution (50 nM final concentration).
- Add 2.5 μ L of 4X ATP solution (10 nM to 0.3 mM final concentrations).
- Cover plate with TopSeal-A and incubate 30 min at 23°C.
- Add 5 μ L of 4X Stop solution* and incubate 5 min at 23°C.
- Add 5 μ L of 4X Detection Mix (2 nM Eu-anti-phospho-tyrosine antibody (PT66) final concentration).
- Cover plate with TopSeal-A and incubate 60 min at 23°C.
- Remove TopSeal-A and read in TR-FRET mode at 665 nm (excitation at 320 or 340 nm).

*Alternatively, the Stop solution and Detection Mix can be premixed and added together to the kinase reaction.

Typical Product Data

JAK3 kinase assay using *ULight*-JAK-1 Peptide and Europium-anti-phospho-tyrosine (PT66) Antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

- | | |
|---|-------------------|
| • Substrate: <i>ULight</i> [™] - JAK-1 (Tyr1023) Peptide | Supplier |
| • Antibody: Eu-anti- phospho-tyrosine antibody (PT66) | Revvity Inc |
| • Kinase: JAK3, active | Revvity Inc |
| • Detection Buffer: LANCE [®] Detection Buffer, 10X | Carna Biosciences |
| • Plate: OptiPlate [™] -384, white | Revvity Inc |
| • TopSeal [™] -A | Revvity Inc |

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Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000 www.revvity.com

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