TECH NOTE *U*-TRF #15

JAK3 Kinase Assay

U*Light*-JAK-1 (Tyr1023) Peptide and Europium-anti-phospho-tyrosine Antibody (PT66)

Two LANCE *Ultra* companion products – two convenient sizes!

U*Light*™-JAK-1 (Tyr1023) Peptide:

- TRF0121-D: 0.5 nmole, 1,000* assay points
- TRF0121-M: 5 nmoles, 10,000* assay points

**0.5 pmol/assay point*

PEPTIDE SEQUENCE:

CAGAGAIETDKEY**Y**TVKD

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

VALIDATED FOR KINASES: ALK, EGFR, EPHB4, FGFR1, FLT1, ITK, JAK1, JAK2, JAK3, LCK, LYNa, PDGFRA, RET, SRC, TIE2, ZAP70

Europium-anti-phospho-tyrosine Antibody (PT66):

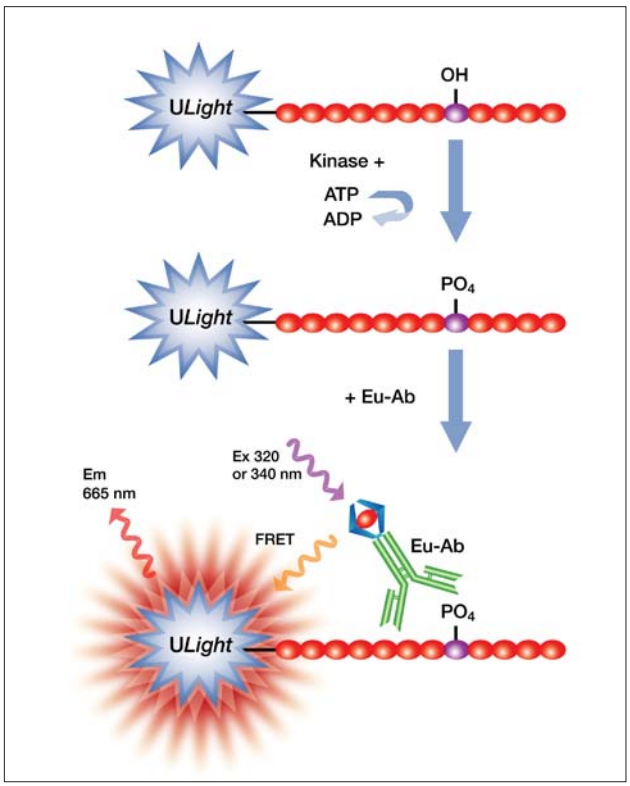
- AD0068: 50 µg, 7,500* assay points
- AD0069: 1 mg, 150,000* assay points

**40 fmol/assay point*

RECOGNIZED MOTIF:

pTyr

Mouse monoclonal antibody directed against phospho-tyrosine.



LANCE *Ultra* Kinase Assays

LANCE® *Ultra* time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W-1024 (Eu), with U*Light*, an innovative small molecular weight acceptor dye with a red-shifted fluorescent emission. In kinase assays, the binding of a Eu-labeled anti-phospho-substrate antibody to the phosphorylated U*Light*-labeled substrate brings donor and acceptor molecules into close proximity.

After irradiation of the kinase reaction at 320 or 340 nm, the energy from the Eu donor is transferred to the U*Light* acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of U*Light*-substrate phosphorylation.

Development of a JAK3 Kinase Assay

Additional reagents

JAK3	Carna # 08-046
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A	PerkinElmer # 6005185

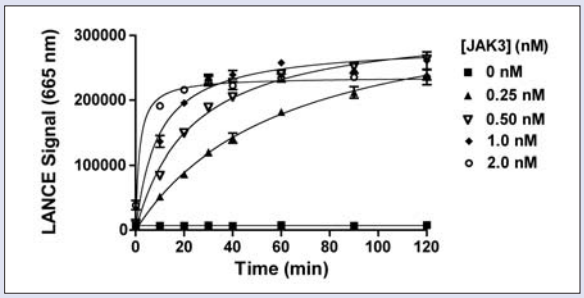
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.

PerkinElmer® precisely.

- Dilute the JAK3 kinase, ATP, inhibitors and U*Light*-JAK-1 (Tyr1023) Peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-tyrosine antibody (PT66) to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of JAK3 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-JAK-1 (Tyr1023) Peptide/ ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A and incubate at room temperature (RT).
- Stop kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 µL of 4X Detection Mix (Eu-anti-phospho–tyrosine Antibody (PT66) at a final concentration of 2 nM).
- Cover with TopSeal-A and incubate for 1 h at RT.
- Remove TopSeal-A and read signal with the EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 nm and emission at 665 nm).

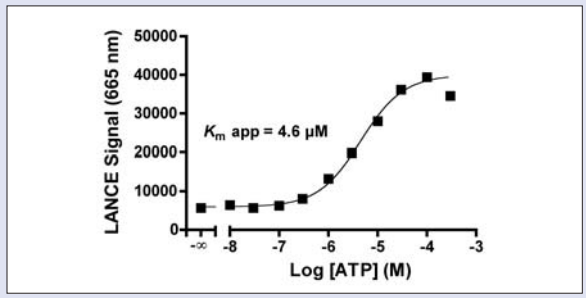
NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course



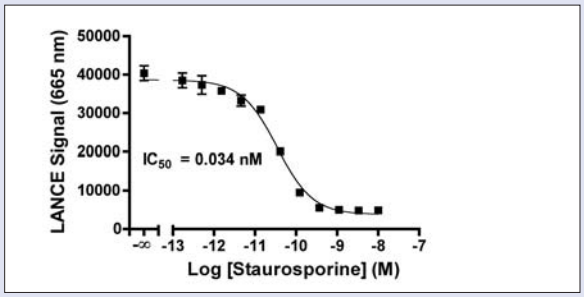
JAK3 enzyme was incubated at concentrations ranging from 0.25 to 2 nM with 50 nM U*Light*-JAK-1 (Tyr1023) Peptide and 20 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



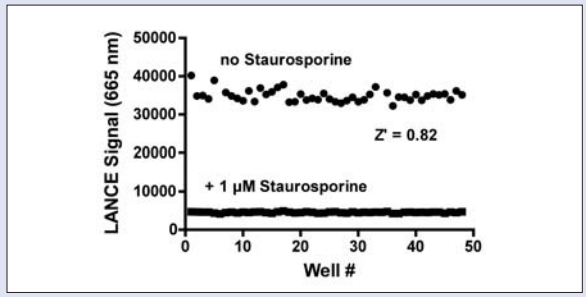
Serial dilutions of ATP ranging from 10 nM to 0.3 mM were added to 0.25 nM JAK3 and 50 nM U*Light*-JAK-1 (Tyr1023) Peptide. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 0.2 pM to 10 nM (final concentrations in 2% DMSO) were incubated with 0.25 nM JAK3, 50 nM U*Light*-JAK-1 (Tyr1023) Peptide and 10 µM ATP. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



JAK3 enzyme at 0.25 nM was incubated with 50 nM U*Light*-JAK-1 (Tyr1023) Peptide and 10 µM ATP with or without 1 µM staurosporine (final concentrations in 2% DMSO). Kinase reactions were terminated after 30 min by the addition of EDTA.

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