

LANCE® Ultra

**ULight™ -labeled Poly GAT**

Product number: TRF0101-D Lot Number: 3267092

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Product Format: TRF0101-D: 1 nmole (100µL)

TRF0101-M: 10 nmoles (1mL)

Manufacturing date: 03/06/2024 Document version: 1

**Product Information**

Phosphorylation Motif: [EAY(1:1:1)]n

Storage Buffer: 50 mM Tris-HCl (pH 7.4), 0.9% NaCl, 0.1% BSA and 0.05% sodium azide as preservative

Molecular Weight: 35 000

Stability: This product is stable for at least **10 months** from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Storage Conditions: Store at -80°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 antibody. We certify that results meet our quality release criteria.

Labeling Ratio: 0.5 (dye molecule/peptide)

Concentration: 350 µg/mL (10 µM)

**Recommended Assay Conditions**

Src kinase assay: ATP titration

## SUGGESTED METHOD:

(Specific applications might require optimization)

### Reagent Preparation

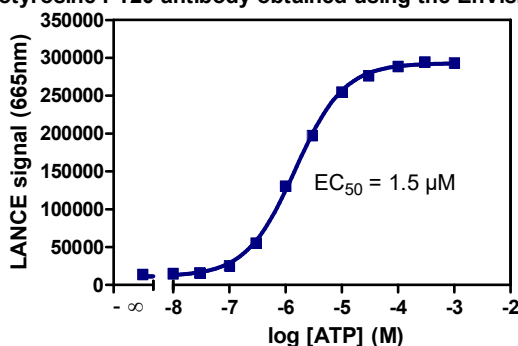
- Prepare 1X Kinase Assay Buffer: 50 mM Tris-HCl pH 7.5, 1 mM EGTA, 10 mM MgCl<sub>2</sub>, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X c-Src enzyme solution: dilute stock enzyme to concentration of 2 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X *ULight*-poly GAT solution: dilute *ULight*-poly GAT to a concentration of 400 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X ATP solution: dilute ATP to concentrations ranging from 40 nM to 4 mM (serial half-log 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 H<sub>2</sub>O.
- Prepare a 4X Stop Solution: prepare a 40 mM EDTA solution in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute the Eu-W1024-labeled PY20 antibody to a concentration of 8 nM in 1X Detection Buffer.

### Protocol

- Pipet 5  $\mu$ L of 2X c-Src enzyme into a 384-well white OptiPlate-384 (1 nM final concentration).
- Add 2.5  $\mu$ L of 4X *ULight*-poly GAT solution (100 nM final concentration).
- Add 2.5  $\mu$ L of 4X ATP solution (10 nM to 1 mM final concentrations).
- Cover plate with TopSeal-A and incubate 60 min at 23°C.
- Add 5  $\mu$ L of 4X Stop Solution and incubate 5 min at 23°C.
- Add 5  $\mu$ L of 4X Detection Mix (2 nM Eu-labeled PY20 antibody final concentration) and mix.
- 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).

## Typical Product Data

Src kinase assay using *ULight*-poly GAT and Eu-anti phosphotyrosine PY20 antibody obtained using the EnVision® Multilabel Reader:



## Suggested Materials

- Substrate: *ULight*<sup>™</sup>-poly Glu, Ala, Tyr (1:1:1)
- Antibody: Eu-W1024 anti-phosphotyrosine (PY20)
- Kinase: c-Src
- Detection Buffer: LANCE<sup>®</sup> Detection Buffer, 10X
- Plate: OptiPlate<sup>™</sup>-384, white

### Supplier

Revvity Inc  
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- TopSeal™-A

Revvity Inc

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TRF0101-R Rev01

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