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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

ULight™-labeled Poly GAT

Product number: TRF0101-D **Lot Number:** 3267092

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

TRF0101-M: 10 nmoles (1mL)

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

Product Information

Phosphorylation Motif: [EA**Y**(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

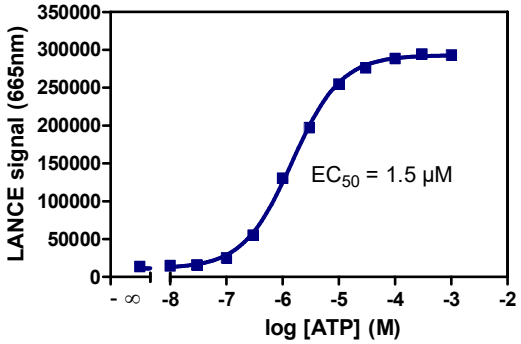
- Prepare 1X Kinase Assay Buffer: 50 mM Tris-HCl pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X c-Src enzyme solution: dilute stock enzyme to concentration of 2 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X U*Light*-poly GAT solution: dilute U*Light*-poly GAT 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_2O.
- 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 µL of 2X c-Src enzyme into a 384-well white OptiPlate-384 (1 nM final concentration).
- Add 2.5 µL of 4X U*Light*-poly GAT solution (100 nM final concentration).
- Add 2.5 µL 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 µL of 4X Stop Solution and incubate 5 min at 23°C.
- Add 5 µL 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

	Supplier
• Substrate: U*Light*™-poly Glu, Ala, Tyr (1:1:1)	Revvity Inc
• Antibody: Eu-W1024 anti-phosphotyrosine (PY20)	Revvity Inc
• Kinase: c-Src	UpState
• Detection Buffer: LANCE® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc

- TopSeal™-A Revvity Inc

TRF0101-R Rev01

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