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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

# *ULight*™-labeled Poly GT

**Product number:** TRF0100-D **Lot Number:** 3304557

**Product Format:** TRF0100-D: 1 nmole (100µL)

TRF0100-M: 10 nmoles (1mL)

**Manufacturing date:** 06/27/2024 **Document version:** 1

## Product Information

**Phosphorylation Motif:** [E**Y**(4:1)]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 **15 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**: 0.3 (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

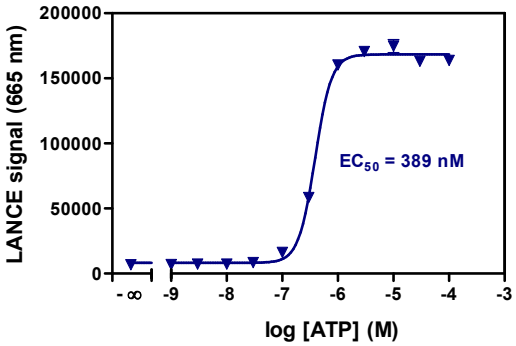
- Prepare 1X Kinase Assay Buffer: 50 mM Tris-HCl pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X c-Src enzyme solution: dilute stock enzyme to concentration of 2 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X U*Light*-poly GT solution: dilute U*Light*-poly GT to 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 GT 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 U*Light*-poly GT and Eu-anti phosphotyrosine PY20 antibody obtained using the EnVision® Multilabel Reader:**




**Suggested Materials**

| | **Supplier** |
|---|---|
| • Substrate: U*Light*™- poly GT (4:1) | 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 |



TRF0100-R Rev01

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