revvity

CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

ULight™-labeled Streptavidin

Product number: TRF0102-R **Lot Number:** 3258514

Product Format: TRF0102-D: 1 nmole (100µL)

TRF0102-M: 10 nmoles (1mL)

TRF0102-R: 100 nmoles (10mL)

Manufacturing date: 01/29/2024 **Document version:** 1

Product Information

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

Molecular Weight: 60 000

Stability: This product is stable for at least **24 months** from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Storage Conditions: Store at 4°C. Store protected from light.

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: 3.1(dye molecule/peptide)
Concentration: 600 µg/mL (10 µM)

Recommended Assay Conditions

Src kinase assay: ATP titration

SUGGESTED METHOD:
(Specific applications might require optimization)

Reagent Preparation

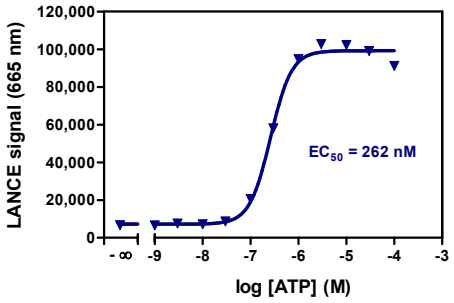
- Prepare 1X Kinase Buffer: 50 mM Tris-HCl pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X c-Src enzyme solution: dilute the enzyme to a concentration of 2 nM in Kinase Buffer. Keep on ice.
- Prepare a 4X mix of biotin-poly GT: dilute biotin-poly GT to a concentration of 400 nM in Kinase Buffer. Keep on ice.
- Prepare a 4X mix of ATP: dilute ATP to concentrations ranging from 40 nM to 4 mM (serial half-log dilutions) in Kinase 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 8 nM and U*Light*-Streptavidin to 200 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 biotin-poly-GT (100 nM final concentration).
- Add 2.5 µL of 4X ATP mix (10 nM to 1 mM final concentrations).
- Cover plate with TopSeal-A and incubate 90 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-W1024-labeled PY20 antibody and 50 nM U*Light*-Streptavidin final concentrations).
- 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*-Streptavidin, biotin poly GT and Eu-PY20 anti phosphotyrosine obtained using the EnVision® Multilabel Reader:



Suggested Materials

	Supplier
• Detection: U*Light*™- Streptavidin	Revvity Inc
• Substrate: biotin-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

TRF0102-R Rev01

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

(800) 762-4000 www.revvity.com