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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

ULight™-labeled TK Peptide

Product number: TRF0127-D **Lot Number:** 3276720

Product Format: TRF0127-D: 0.5 nmole (100µL)

TRF0127-M: 5 nmoles (1mL)

Manufacturing date: 3/20/2024 **Document version:** 1

Product Information

Phosphorylation Motif: Synthetic peptide containing motifs for Tyr kinase phosphorylation

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

Molecular Weight: 4 232

Stability: This product is stable for at least **12 months** from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Storage Conditions: Store at -20°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 protein. We certify that results meet our quality release criteria.

Labeling Ratio: 1.0 (dye molecule/peptide)
Concentration: 21.2 µg/mL (5 µM)

Recommended Assay Conditions

EPHA4 kinase: ATP titration

SUGGESTED METHOD:
(Specific applications might require optimization)

Reagent Preparation

- Prepare 1X Kinase Assay Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X EphA4 solution: dilute enzyme to a concentration of 240 pM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X U*Light*-TK solution: dilute U*Light*-TK to a concentration of 200 nM in Kinase Assay Buffer.
- 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*: dilute EDTA to a concentration of 40 mM in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute Europium-anti-phosphotyrosine (PT66) to a concentration of 8 nM in 1X Detection Buffer.

Protocol

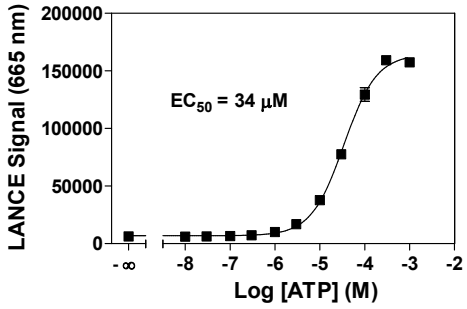
- Pipet 5 µL of 2X EphA4 solution into a 384-well white OptiPlate-384 (120 pM final concentration).

- Add 2.5 µL of 4X U*Light*-TK solution (50 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-anti-phosphotyrosine (PT66) antibody final concentration).
- 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).

**Alternatively, the Stop solution and Detection Mix can be premixed just before use and added together to the kinase reaction.*

Typical Product Data

EphA4 kinase assay using U*Light*-TK Peptide and Eu-anti-phosphotyrosine (PT66) Antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

	Supplier
• Substrate: U*Light*™- TK Peptide	Revvity Inc
• Antibody: Eu-anti- anti-phosphotyrosine (PT66)	Revvity Inc
• Kinase: EphA4, active	Carna Biosciences
• Detection Buffer: LANCE® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc

TRF0127-R Rev01

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