

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<sub>2</sub>, 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 ULight-TK solution: dilute ULight-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<sub>2</sub>O.
- 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

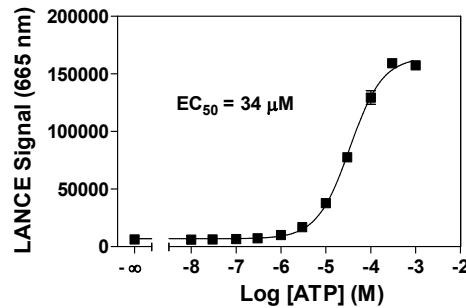
- Pipet 5 µL of 2X EphA4 solution into a 384-well white OptiPlate-384 (120 pM final concentration).

- Add 2.5  $\mu$ L of 4X *ULight*-TK solution (50 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-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 *ULight*-TK Peptide and Eu-anti-phosphotyrosine (PT66) Antibody obtained using the EnVision® Multilabel Reader:**



## Suggested Materials

- Substrate: *ULight*<sup>™</sup>- TK Peptide
- Antibody: Eu-anti- anti-phosphotyrosine (PT66)
- Kinase: EphA4, active
- Detection Buffer: LANCE<sup>®</sup> Detection Buffer, 10X
- Plate: OptiPlate<sup>™</sup>-384, white
- TopSeal<sup>™</sup>-A

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TRF0127-R Rev01

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