

Caution: For Laboratory Use. A product for research purposes only

ULight[™] -labeled Streptavidin

Product No.: TRF0102-D / TRF0102-M / TRF0102-R

Lot No.: 676-537-A

Material Provided

Format:

| | |
|-----------|-------------------------------------|
| TRF0102-D | 1 nmole (1 000 assay points*) |
| TRF0102-M | 10 nmoles (10 000 assay points*) |
| TRF0102-R | 100 nmoles (100 000 assay points*) |

*Assuming 1 pmol/ assay point

Volume: 100 µL (TRF0102-D), 1 mL (TRF0102-M) or 10 mL (TRF0102-R)

Manufacturing Date: **June 22, 2011**

Product Information

Molecular Weight: 60 000

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

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.

Safety Note: The storage buffer contains sodium azide (NaN₃) as a preservative. Disposal of all waste should be in accordance with local regulations.

Quality Control

The QC release specifications are based on spectrophotometric analysis of the labeled protein. We certify that these results meet our quality release criteria.

Labeling Ratio: 2.9 (dye molecule/protein)

Concentration: 600 µg/mL (10 µM)

Recommended Assay Conditions

Src Kinase assay: ATP titration

Reagent Preparation:

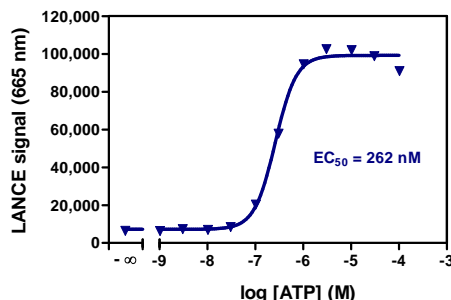
- Prepare 1X Kinase Buffer: 50 mM Tris-HCl pH 7.5, 1 mM EGTA, 10 mM MgCl₂, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X c-Src enzyme solution: dilute the enzyme to a concentration of 2 nM in Kinase Buffer. Keep on ice.
- Prepare a 4X mix of biotin-poly GT: dilute biotin-poly GT to a concentration of 400 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 H₂O.
- 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 *ULight*-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 *ULight*-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 ATP Titration Data

Src kinase assay using *ULight*-Streptavidin, biotin poly GT and Eu-PY20 anti phosphotyrosine obtained using the EnVision® Multilabel Reader:



Suggested Materials

| | Supplier | Cat. No. |
|--|-------------|----------------|
| • Detection: <i>ULight</i> [™] - Streptavidin | PerkinElmer | TRF0102 |
| • Substrate: biotin-poly GT (4:1) | PerkinElmer | Custom product |
| • Antibody: Eu-W1024 anti-phosphotyrosine (PY20) | PerkinElmer | AD0066 |
| • Kinase: c-Src | Upstate | 14-326 |
| • Detection Buffer: LANCE [®] Detection Buffer, 10X | PerkinElmer | CR97-100 |
| • Plate: OptiPlate [™] -384, white | PerkinElmer | 6007299 |
| • TopSeal [™] : TopSeal-A | PerkinElmer | 6005185 |

Please visit our website for additional resource:

www.perkinelmer.com/LANCE

This product is not for resale or distribution except by authorized distributors.

Ulight[™] products may be covered by the following patents: US 7,250,517 and EP07102880.

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