

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

ULight[™]-labeled MBP peptide

Product No.: TRF0109-M

Lot No.: 2703414

Material Provided

Format: TRF0109-D 0.5 nmole (1 000 assay points*)
TRF0109-M 5 nmoles (10 000 assay points*)
*Assuming 0.5 pmol/ assay point

Volume: 100 µL (TRF0109-D) or 1 mL (TFR0109-M)

Manufacturing Date: April 6, 2020

Product Information

Phosphorylation Motif: VTPRTTPPP
Human Myelin Basic Protein phosphorylation site: Thr232 (Swiss-Prot: P02686); corresponds to Thr98 in other isoforms or species.

Molecular Weight: 2 808

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 **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.

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 peptide. We certify that these results meet our quality release criteria.

Labeling Ratio: 1.0 (dye molecule/peptide)

Concentration: 14.05 µg/mL (5 µM)

Recommended Assay Conditions

ERK1 kinase: ATP titration

Reagent Preparation:

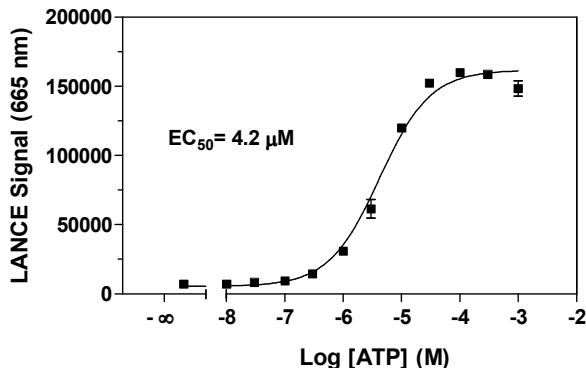
- Prepare 1X Kinase Assay Buffer: 50 mM Tris pH 7.5, 1 mM EGTA, 10 mM MgCl₂, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X Erk1 solution: dilute the enzyme to a concentration of 2 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X mix of *ULight*-MBP: dilute *ULight*-MBP to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 4X mix of ATP: 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 H₂O.
- Prepare a 4X Stop Solution: prepare a 40 mM EDTA solution in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute the Eu-anti-phospho-MBP antibody to a concentration of 8 nM in 1X Detection Buffer.

Protocol:

- Pipet 5 µL of 2X Erk1 solution into a 384-well white OptiPlate™-384 (1 nM final concentration).
- Add 2.5 µL of 4X *ULight*-MBP (50 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 Detection Mix (2 nM Eu-anti-phospho-MBP 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 ATP Titration Data

Erk1 kinase assay using *ULight*-MBP and Eu-anti-phospho-MBP antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

| | Supplier | Cat. No. |
|--|-------------|----------|
| • Substrate: <i>ULight</i> [™] - MBP peptide | PerkinElmer | TRF0109 |
| • Antibody: Eu-anti- phospho-Myelin Basic Protein (MBP) | PerkinElmer | TRF0201 |
| • Kinase: MAP Kinase 1/Erk1, active | Upstate | 14-439 |
| • Detection Buffer: LANCE [®] Detection Buffer, 10X | PerkinElmer | CR97-100 |
| • Plate: OptiPlate [™] -384, white | PerkinElmer | 6007299 |
| • TopSeal [™] : TopSeal-A | PerkinElmer | 6050195 |

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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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com



For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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