TECHNICAL DATA SHEET

LANCE® *Ultra* Research Reagents

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

ULight™-labeled MBP peptide

Product No.: TRF0109-M

Lot No.: 2927726

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 November 15, 2021

Product Information

Phosphorylation Motif: VTPR**T**PPP
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 (NaN_3) 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)

PerkinElmer® For the Better

Recommended Assay Conditions

ERK1 kinase: ATP titration

Reagent Preparation:

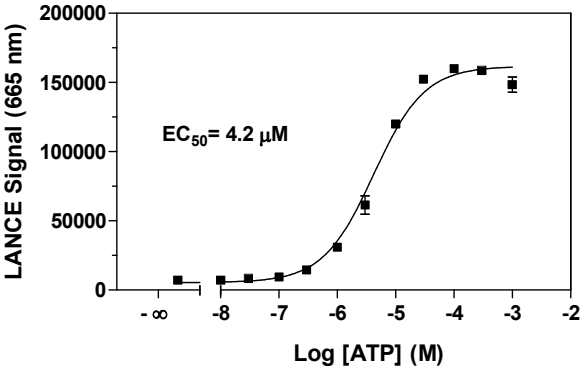
- Prepare 1X Kinase Assay Buffer: 50 mM Tris pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X Erk1 solution: dilute the enzyme to a concentration of 2 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X mix of U*Light*-MBP: dilute U*Light*-MBP to a concentration of 200 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 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-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 U*Light*-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 U*Light*-MBP and Eu-anti-phospho-MBP antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

	Supplier	Cat. No.
• Substrate: U*Light*™- 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

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