Research use only. Not for use in diagnostic procedures.

LANCE® Ultra

Eu-W1024 labeled Anti-phospho-Myelin Basic Protein (MBP)

Antibody

Product number: TRF0201-C **Lot Number:** 3269460

Product Format: TRF0201-C: 1.7 μg

Manufacturing date: January 3, 2024 Document version: 1

Product Information

Antibody: Europium-labeled mouse monoclonal antibody recognizing phospho-Thr232* in human Myelin

Basic Protein (MBP; Swis-Prot: P02686).

*corresponds to Thr98 in other isoforms or species

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

Molecular Weight: 160 000

Stability: This product is stable for at least 18 months from the manufacturing date when stored in its

original packaging and at the recommended storage conditions.

Storage Conditions: Store at 4°C

Quality Control

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

Labeling Ratio: 7.07

Concentration: $100 \mu g/ml (0.625 \mu M)$

Recommended Assay Conditions

ERK1 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 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.
- \bullet 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.

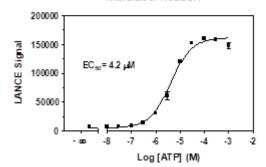
• Prepare a 4X Detection Mix: dilute the Eu-anti-phospho-MBP antibody to a concentration of 8 nM in 1X Detection Buffer.

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 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 Product Data

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



Suggested Materials

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|---|--|--------------|
| • | Substrate: U <i>Light</i> ™- MBP Peptide | Revvity Inc |
| • | Antibody: Eu- phospho-Myelin Basic Protein (MBP) | Revvity Inc |
| • | Kinase: MAP Kinase 1/Erk1, active | Upstate |
| • | Detection Buffer: LANCE® Detection Buffer, 10X | Revvity Inc |
| • | Plate: OptiPlate™-384, white | Revvity Inc |
| • | TopSeal™-A | Revvity Inc. |

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