CERTIFICATE OF ANALYSIS

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 µg/ml (0.625 µM)

Recommended Assay Conditions

ERK1 KINASE: ATP TITRATION

SUGGESTED METHOD:
(Specific applications might require optimization)

Reagent Preparation

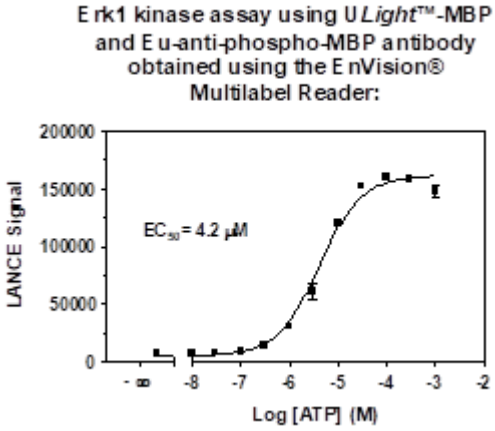
- Prepare 1X Kinase Assay Buffer: 50 mM Hepes pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X Erk1 solution: dilute the enzyme to a concentration of 2 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X mix of U*Light*™-MBP: dilute U*Light*™-MBP to a concentration of 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 Product Data



Suggested Materials

	Supplier
• Substrate: U*Light*™- 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

TRF0201-C-R Rev01

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000 www.revvity.com

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