

LANCE® *Ultra*

Eu-W1024 labeled Anti-V5 Antibody

Product number: AD0277 Lot Number: 3266961

Product Format: AD0276: 10 µg
AD0277: 50 µg
AD0278: 1 mg

Manufacturing date: 12/01/2023 Document version: 1

Product Information

Application: Eu-W1024 has been conjugated to anti-V5 antibody. This antibody recognizes V5 peptide either freely or conjugated to molecules such as proteins or DNA. This toolbox may be used as a secondary antibody to primary antibodies labeled with V5 in TR-FRET assays.

Storage: Store product in the dark at 4 °C.

Stability: This kit is stable for at least 6 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

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: 7.4
Concentration: 100 µg/mL (0.625 µM)

Description of the LANCE *Ultra* Assay

LANCE® and LANCE® (Lanthanide chelate excite) *Ultra* are our TR-FRET (time-resolved fluorescence resonance energy transfer), homogeneous (no wash) technologies. One antibody of interest is labeled with a donor fluorophore (a LANCE Europium chelate) and the second molecule is labeled with an acceptor fluorophore [ULight™ dye]. Upon excitation at 320 or 340 nm, energy can be transferred from the donor Europium chelate to the acceptor fluorophore if sufficiently close for FRET (~10 nm). This results in the emission of light at 665 nm.

Recommended Assay Conditions

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the signal.

Specific additional required reagents and materials:

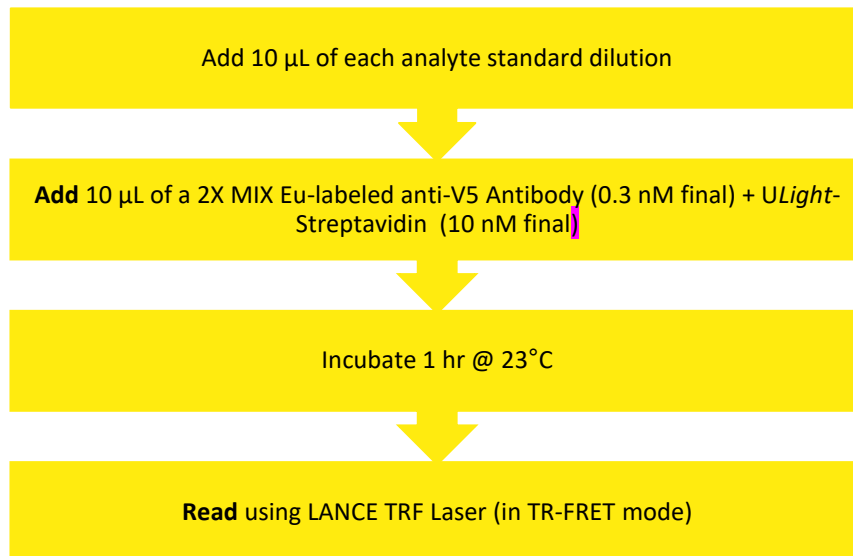
The following materials are recommended:

Item	Suggested source
OpitPlate-96 or OptiPlate-384	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
Multilabel Plate Reader equipped with TR-FRET option, such as the EnVision®	Revvity Inc.

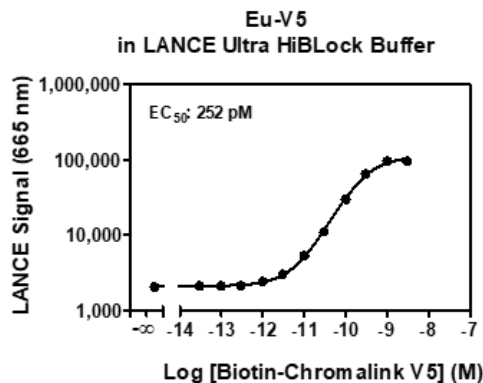
Example: Eu-W1024 Anti-V5 LANCE *Ultra* Assay

Protocol

1. Prepare 1X *Ultra* HiBlock Buffer: Add 2 mL of 5X *Ultra* HiBlock Buffer to 8 mL H₂O.
2. Prepare Biotin-V5 probe standard dilutions:
 - a. Add 5 µL of 500 µM Biotin-V5 stock solution in 495 µL of *Ultra* HiBlock Buffer (5 µM)
 - b. Add 5 µL of 5 µM dilution to 495 µL of 1X *Ultra* HiBlock Buffer (50 nM)
 - c. Prepare standard dilutions in 1X *Ultra* HiBlock Buffer (6 nM – 0.06 pM)
3. Prepare 500 nM *ULight*-Streptavidin:
 - a. Add 5 µL of *ULight*-Streptavidin (10 µM) and 1.3 µL of BSA 7.5% to 93.7 µL of 1X TSA buffer (50 mM Tris-HCl – 150 mM NaCl – 0.05% sodium azide) pH 7.4
4. Preparation of 2X MIX Eu-labeled anti-V5 Antibody (0.6 nM) + *ULight*-Streptavidin (20 nM):
 - a. Add 3 µL of 500 nM Eu-W1024 labeled Anti-V5 to 27 µL of 1X *Ultra* HiBlock Buffer (50 nM).
 - b. Add 6 µL of 50 nM Eu-labeled anti-V5 Antibody and 20 µL of 500 nM *ULight*-Streptavidin to 474 µL of *Ultra* HiBlock Buffer.
 - c. Prepare just before use



Typical Product Data



Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec).
- Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H₂O (18 MΩ•cm) to dilute Buffer.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. LANCE *Ultra* TR-FRET assays cannot be read with the TopSeal-A Film attached. Please remove before reading.
- LANCE signal is detected using a Multilabel Reader equipped with the TR-FRET. Use an excitation wavelength of 320 or 340 nm to excite the LANCE Europium chelate. We recommend you read this assay in dual emission mode, detecting both the emission from the Europium donor fluorophore at 615 nm, and the acceptor fluorophore (at 665 nm for *ULight* dye). The raw FRET signal at 665 nm can be used to process your data.

Please visit our website for additional information on LANCE *Ultra* technology at www.revvy.com

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