TECHNICAL DATA SHEET

LANCE Ultra® Research Reagents

Eu-W1024 labeled Anti-FLAG LANCE Ultra Toolbox

Product number: AD0273 / AD0274 / AD0275

Lot number: 2568246

Manufacturing date: April 23, 2019

Research Use Only. Not for use in diagnostic procedures.

Material Provided

Format:

AD0273 10 µg
AD0274 50 µg
AD0275 1 mg

Manufacturing Date: April 23, 2019

Product Information

Application: Eu-W1024 has been conjugated to anti-FLAG antibody. The mouse monoclonal recognizes the FLAG sequence at the N-terminus, Met N-terminus, C-terminus, and internal sites. This toolbox can be used to capture FLAG-tagged proteins and peptides,

Storage: Store kit in the dark at +4˚C.

Stability: This kit is stable for at least 12 months from the manufacturing date 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: 6.9

Concentration: 0.5 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 [U*Light*™ 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:

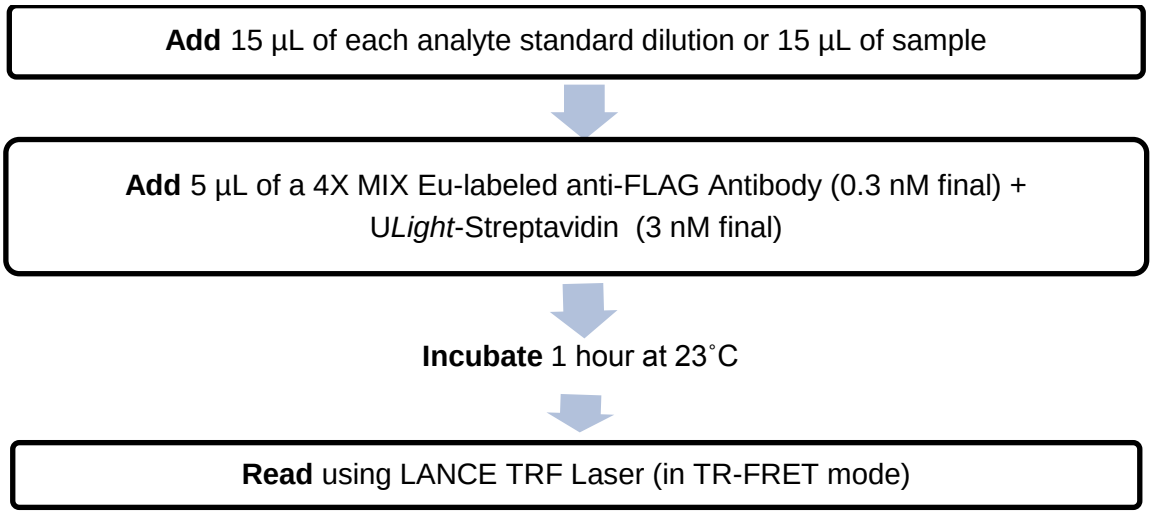
Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer Inc.	6050185
Optiplate 96 or 384-well plate	PerkinElmer Inc.	6005290 (96) 6007299 (384)
VICTOR X, ViewLux, EnVision or EnSpire Multilabel Plate Reader equipped with TR-FRET option	PerkinElmer Inc.	-

Example: **Eu-W1024 Anti-FLAG LANCE *Ultra* QC Assay**

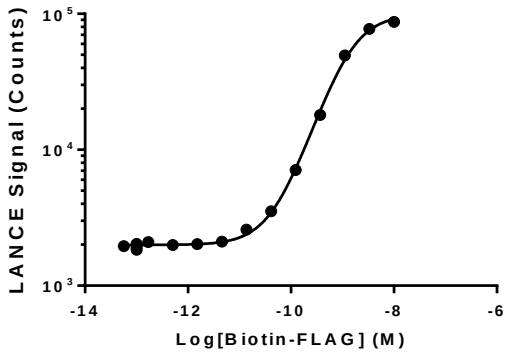
Reagents:

1. Prepare 1X Buffer: Add 2 mL of 5X *Ultra* HiBlock Buffer to 8 mL H_2O.
2. Prepare Chromalink Biotin-FLAG probe standard dilutions:
    a. Dilute Biotin-FLAG Probe to 0.1 µM with 1X *Ultra* HiBlock Buffer.
    b. Prepare standard dilutions in 1X Ultra HiBlock Buffer (0.01 µM – 0.03 pM)
3. Prepare 500 nM U*Light*-Streptavidin:
    a. Add 5 µL of U*Light*-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 4X MIX Eu-labeled anti-FLAG Antibody (1.2 nM) + U*Light*-Streptavidin (12 nM):
    a. Add 2.4 µL of 500 nM Eu-labeled anti-FLAG Antibody and 24 µL of 500 nM U*Light*-Streptavidin to 973.6 µL of 1X *Ultra* HiBlock Buffer.
    b. Prepare just before use

Protocol:



Typical 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 H_2O (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 VICTOR X, ViewLux, EnVision or EnSpire 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 U*Light* dye). The raw FRET signal at 665 nm can be used to process your data.
- Signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.

Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your LANCE Ultra Assay kit at:

http://www.perkinelmer.com/Resources/TechnicalResources/ApplicationSupportKnowledgebase/LANCE/lance.xhtml

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