TECHNICAL DATA SHEET

LANCE® *Ultra* Research Reagents

Anti-Fluorescein isothiocyanate (FITC) U*Light* LANCE *Ultra* Toolbox

Product number: TRF503D / TRF503M / TRF503R

Lot number: 2782629

Manufacturing date: October 26, 2020

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

Material Provided

Format:

TRF503D 0.2 nmole (1 000 assay points*)
TRF503M 2 nmoles (10 000 assay points*)
TRF503R 20 nmoles (100 000 assay points*)

*Assuming 0.2 pmol/ assay point

Volume: 400 µL (TRF503D), 4 x 1 mL (TRF503M) or 4 x 10 mL (TRF503R)

Manufacturing Date:

Product Information

Application: U*Light* has been conjugated to anti-fluorescein antibody. This antibody recognizes fluorescein isothiocyanate 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 FITC in TR-FRET assays.

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

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Stability: This kit is stable for at least 6 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: 4.7

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: **Anti-FITC *ULight LANCE Ultra* QC Assay**

Reagents:

1. Prepare 1x Buffer: Add 2 mL of 5X PBS + 0.1% Tween 20 to 8 mL H_2O.
2. Prepare Biotin-FITC probe standard dilutions:
    a. Dissolve Biotin-FITC Probe in DMSO.
    b. Dilute Biotin-FITC to 10 µM with Milli-Q water
    c. Dilute Biotin-FITC Probe to 1 µM with 1X PBS + 0.1% Tween 20
    d. Prepare standard dilutions in 1X PBS + 0.1% Tween 20 (0.1 µM – 1 pM)
3. Prepare 500 nM Eu-W1024 Streptavidin:
    a. Dissolve Eu-W1024 Streptavidin (1 mg) to 500 nM with 1X TSA buffer (50 mM Tris-HCl – 150 mM NaCl – 0.05% sodium azide) pH 7.4
4. Preparation of 4X MIX Eu-W1024 Streptavidin (4 nM) + U*Light* labeled anti-FITC Antibody (40 nM):
    a. Add 4 µL of 500 nM Eu-W1024 Streptavidin and 40 µL of 500 nM *ULight* labeled anti-FITC Antibody and to 456 µL of PBS + 0.1% Tween 20.
    b. Prepare just before use

Protocol:

Add 15 µL of each analyte standard dilution or 15 µL of sample

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Add 5 µL of a Eu-W1024 Streptavidin (1 nM final) +
U*Light* labeled anti-FITC Antibody (10 nM final)

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Incubate 1 hour at 23˚C

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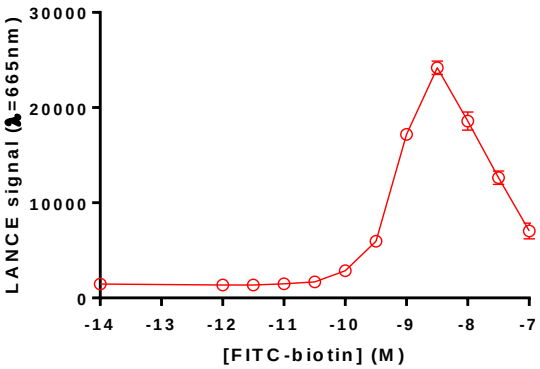
Read using LANCE TRF Laser (in TR-FRET mode)

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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This product is not for resale or distribution except by authorized distributors.

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