TECHNICAL DATA SHEET

LANCE® *Ultra* Research Reagents

Anti-Mouse Immunoglobulin G (mIgG) U*Light* LANCE *Ultra* Toolbox

Product number: TRF501D / TRF501M / TRF501R

Lot number: 2801690

Manufacturing date: October 5, 2020

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

Material Provided

Format:

TRF501D 0.2 nmole (1 000 assay points*)
TRF501M 2 nmoles (10 000 assay points*)
TRF501R 20 nmoles (100 000 assay points*)

*Assuming 0.2 pmol/ assay point

Volume: 400 µL (TRF501D), 4 x 1 mL (TRF501M) or 4 x 10 mL (TRF501R)

Product Information

Application: U*Light* has been conjugated to anti-mouse IgG antibody. This antibody recognizes mouse immunoglobulins without considerations of isoforms or chain type. This toolbox can be used to either detect mouse immunoglobulins in samples, or may be used as a secondary antibody to mouse antibodies in TR-FRET assays, as mouse are the most commonly encountered monoclonal antibodies in detection assays..

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

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: 5.0 ULight/Protein

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-mouse IgG U*Light* LANCE *Ultra* Assay

Reagents:

1. Prepare 1x Buffer: Add 2 mL of 5X PBS + 0.1% Tween 20 to 8 mL H_2O.
2. Prepare Biotin-mouse IgG probe standard dilutions: Dilute Biotin-mouse IgG Probe to 1µM with 1X PBS + 0.1% Tween 20
3. Dilute 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

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-mouse IgG Antibody (10 nM final)

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Incubate 1 hour at 23˚C

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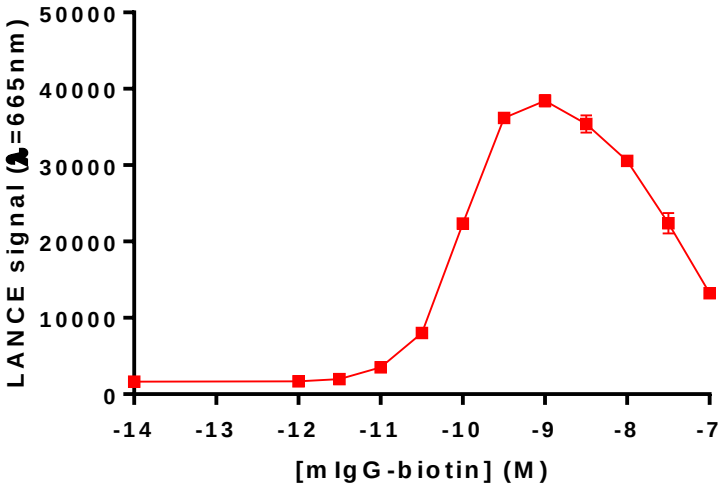
Read using LANCE TRF Laser (in TR-FRET mode)

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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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs