TECHNICAL DATA SHEET

LANCE® *Ultra* Research Reagents

Research Use Only. Not for use in diagnostic procedures.

TREM2 LANCE *Ultra* Cellular Detection Kit

Product number: TRF4030

Contents

○ Product Information

Application: This kit is designed for the detection of TREM2 in cell supernatants using a homogeneous LANCE *Ultra* assay (no wash steps).

Typical Performance (Undiluted positive control supernatant versus Buffer): Signal/Background: 6.7

Z': 0.83

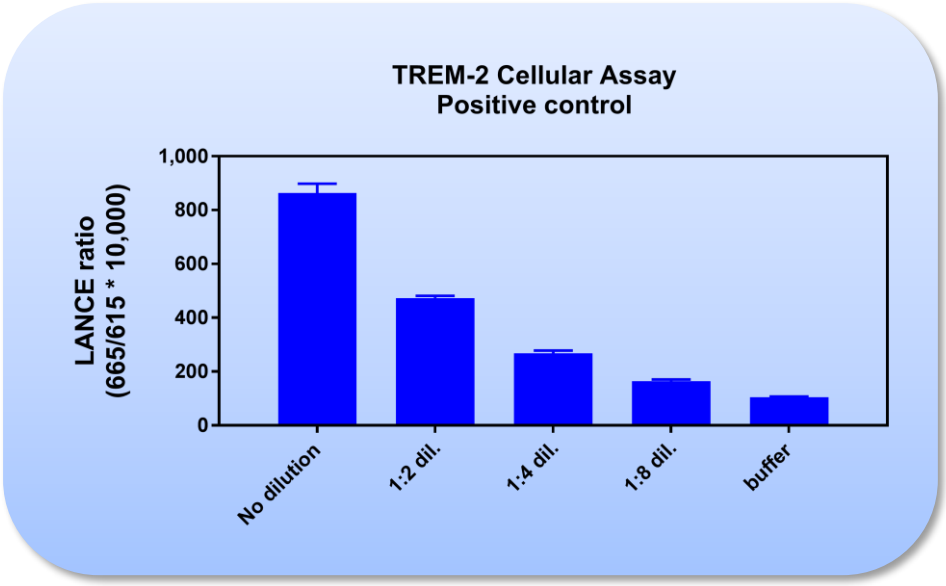


Figure 1. Typical positive control supernatant (THP-1 cells stimulated with PMA) diluted in buffer. The data was generated using a white Optiplate™-384 microplate and read on an EnVision™ Multilabel Plate Reader equipped with TR-FRET laser option. Total signal, signal/background window, and sensitivity may vary with other instruments. Positive control supernatant is not supplied with the kit and must be purchased separately.

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

Lot to lot consistency is confirmed in a LANCE *Ultra* assay. S/B and Z' were measured on the EnVision Multilabel Plate Reader equipped with TR-FRET laser option. We certify that these results meet our quality release criteria. Maximum counts, Signal/Background, and Z' values may vary between lots and instrument used. For quality control purposes, Z' is calculated by comparing 12 replicates of undiluted positive supernatants versus 12 replicates of lysis buffer. Data is calculated and presented ratiometrically by dividing the signal at 665 nm by the signal at 615 nm and multiplied by 10,000.

Analyte of Interest

TREM2 is a transmembrane molecule expressed on myeloid cells. It acts as the receptor for an unknown ligand to activate myeloid cells such as dendritic cells, increasing phagocytic activity. Recently, TREM2 has been shown to be involved in neurodegenerative diseases such as ataxia, early dementia and Alzeimer's disease. Elevated levels of TREM2 have been noted in cerebrospinal fluid as a response to Alzeimer's disease.

Description of the LANCE *Ultra* Assay

LANCE® and LANCE® (Lanthanide chelate excite) *Ultra* are homogeneous (no wash) TR-FRET (time-resolved fluorescence resonance energy transfer) technologies. One antibody of interest is labeled with a donor fluorophore (a LANCE Europium chelate) and the second antibody 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. Data is represented as ratiometric (665/615 nm X 10,000).

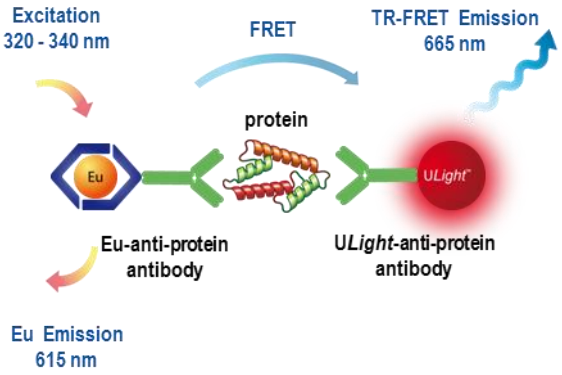


Figure 2. LANCE assay principle

○ Kit Content: Reagents and Materials

Kit components	TRF4030C (500 assay points**)	TRF4030M (10 000 assay points**)
LANCE *Ultra* Eu-labeled Anti-TREM2 Antibody stored in TSA, 0.1% BSA	10 µL @ 500 nM (1 clear tube, yellow cap)	200 µL @ 500 nM (1 clear tube, yellow cap)
LANCE *Ultra* U*Light*-labeled Anti-TREM2 Antibody stored in TSA, 0.1% BSA	100 µL @ 500 nM (1 brown tube, blue cap)	2 X 1000 µL @ 500 nM (2 brown tubes, green caps)
LANCE Detection Buffer (10X) *	1.8 mL, 1 small bottle	250 mL, 1 large bottle
LANCE *Ultra* Lysis Buffer 1 (5X) *	2 mL, 1 small bottle	100 mL, 1 large bottle

* Extra detection buffer can be ordered separately (cat # CR97-100C: 1.8 mL or cat # CR97-100: 250 mL). Extra Lysis Buffer can be ordered separately (cat # TRF001C: 10 mL or cat # TRF001F: 100 mL).

** The number of assay points is based on an assay volume of 20 µL in 384-well assay plates using the kit components at the recommended concentrations.

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 #
VICTOR Nivo™, EnVision, EnSight™, or any Multilabel Plate Reader equipped with TR-FRET option	PerkinElmer Inc.	Please consult our website
TopSeal-A PLUS Adhesive Sealing Film	PerkinElmer Inc.	6050185
Tissue culture treated clear SpectraPlates™, for culturing cells when using the 2-plate protocol	PerkinElmer Inc.	6005650
White OptiPlate-384, for LANCE *Ultra* detection assays when using the 2-plate protocol	PerkinElmer Inc.	6007290
White CulturPlate-384 when using the 1-plate protocol	PerkinElmer Inc.	6007680
Positive Control Cell Supernatant (THP-1 cells treated with PMA)	PerkinElmer Inc.	TRF4030S

○ Recommendations

General 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.
- Re-suspend all reagents by vortexing before use.
- Centrifuge all tubes before use to improve recovery of content (2000x g, 10-15 sec).
- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute Detection and Lysis Buffers.
- When diluting the 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 can be detected using any multimode plate reader equipped with TR-FRET capabilities. Recommended readers are the EnVision, VICTOR NIVO, or EnSight Multilabel Reader equipped with 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 665/615 nm x 10,000 calculation is used to process your data.
- Signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperatures should be used for each plate.
- The representative data shown in this technical data sheet are provided for information only.

Cell Handling recommendations:

- Evaporation can be problematic with cells cultured in microtiter plates. For overnight incubation, it is recommended to add warm PBS or sterile water to unused wells. For longer incubation periods, a sterile breathable sealing membrane (Corning, cat. #3345) can be used to cover the plate. Alternatively, cells can be cultured in larger wells, and/or in a larger volume of culture medium.
- Phosphatase Inhibitors such as NaF and activated Na_3VO_4 can be added to lysis buffers to protect kinases without affecting LANCE detection.
- A starving step with serum-free medium may be required depending on your target/cell line and should be evaluated in a separate experiment.
- For 2-plate protocols with adherent cells: cell seeding densities of 40K cells/well are usually sufficient for most cell lines. However, optimization of cell seeding density is recommended.
- For 1-plate protocols with suspension cells: Cell seeding densities of 100K cells/well are usually sufficient for most cell lines. However, optimization of cell seeding density is recommended.

○ Assay Procedure

		Volume			
Format	**# of data points**	**Final**	**Sample**	**Eu-Antibody/U*Light*-Antibody MIX**	**Plate recommendation**
TRF4030C	250	40 µL	30 µL	10 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	20 µL	15 µL	5 µL	White OptiPlate-384 (cat # 6007290) White CulturPlate-384 (cat # 6007680)
	1 250	8 µL	6 µL	2 µL	ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290) White CulturPlate-384 (cat # 6007680)
	2 500	4 µL	3 µL	1 µL	White OptiPlate-1536 (cat # 6004290)
TRF4030M	10 000	20 µL	15 µL	5 µL	White OptiPlate-384 (cat # 6007290) White CulturPlate-384 (cat # 6007680)
	25 000	8 µL	6 µL	2 µL	ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290) White CulturPlate-384 (cat # 6007680)
	50 000	4 µL	3 µL	1 µL	White OptiPlate-1536 (cat # 6004290)

Reagent Preparation:

1) Preparation of 1X LANCE Detection Buffer and 1X LANCE *Ultra* Lysis Buffer 1:

   a. Add 1 mL of 10X LANCE Detection Buffer to 9 mL H_2O.
   b. Add 2 mL of 5X LANCE *Ultra* Lysis Buffer 1 to 9 mL H_2O.

2) If necessary, dilute supernatant sample with 1X LANCE *Ultra* Lysis Buffer 1 to desired concentration.

3) Preparation of 4X MIX Eu-labeled anti-TREM2 Antibody (2 nM) + U*Light* labeled anti-TREM2 Antibody (20 nM):

   a. Prepare just before use.
   b. Add 10 µL of 500 nM Eu-labeled anti-TREM2 Antibody and 100 µL of 500 nM U*Light*-labeled anti-TREM2 Antibody to 2390 µL of LANCE Detection Buffer

4) In a white Optiplate (384 wells):

Add 15 µL of supernatant sample

↓

Add 5 µL of a 4X MIX Eu-labeled anti-TREM2 Antibody (0.5 nM final) + U*Light* labeled anti-TREM2 Antibody (5 nM final)

↓

Incubate 4 hours at 23˚C*

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

***In order to reduce evaporation, we recommend covering the OptiPlate with TopSeal-A PLUS during the incubation. Longer incubation times can be used and in some cases may improve assay signal/background.**

Important: LANCE signal is detected using an EnVision Multilabel Reader equipped with a TR-FRET laser. 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). Data is calculated and presented ratiometrically by dividing the signal at 665 nm by the signal at 615 nm and multiplying by 10,000.

○ Data Analysis

- Data is represented ratiometrically. Divide the signal at 665 nm by the signal at 615 nm and multiply by 10,000.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a 1/Y2 data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).

○ Assay Performance Characteristics

LANCE Ultra assay performance described below was determined using the 2-plate protocol.

Dose Response:

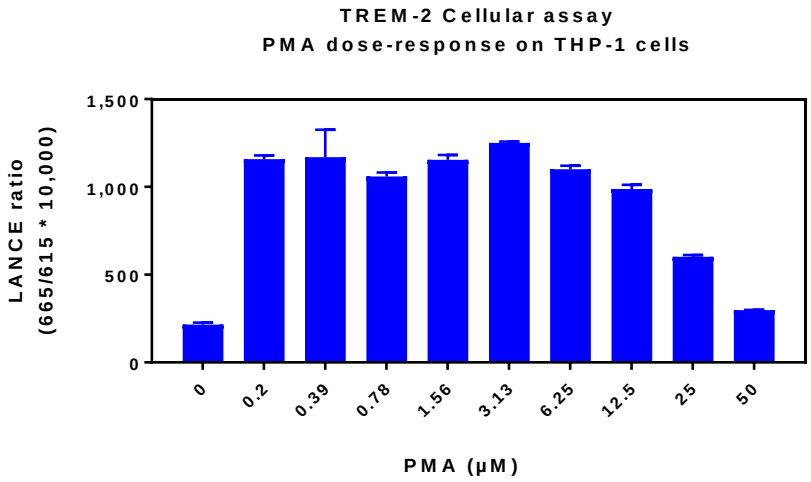
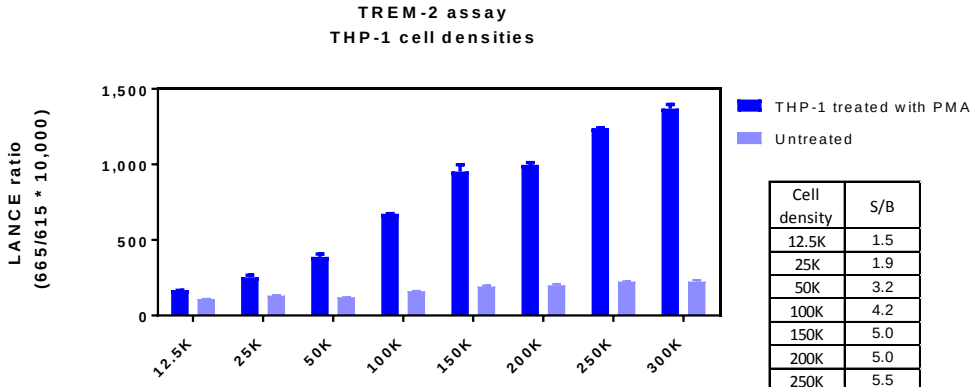


Figure 3: Dose Response Curve THP-1 cells treated with PMA. THP-1 cells (300K/well) in 50 µL of RPMI + 10% FBS were treated with increasing concentrations of PMA over 3 days at room temperature before supernatants were collected and centrifuge for 10 minutes.

Cell Densities:



Cell density	S/B
12.5K	1.5
25K	1.9
50K	3.2
100K	4.2
150K	5.0
200K	5.0
250K	5.5
300K	6.1

Figure 4: Cell Density Effect of THP-1 cells treated with PMA. THP-1 cells at various densities in 50 µL of RPMI + 10% FBS were treated with 6.25 µM of PMA over 3 days at room temperature before supernatants were collected and centrifuge for 10 minutes.

○ Additional Resources

For more information on optimizing LANCE Ultra Cellular Detection Assays follow the link below:

http://www.perkinelmer.com/LANCECellGuide

You will find additional information regarding LANCE *Ultra* Assays at:

http://www.perkinelmer.com/askLANCE

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