TECHNICAL DATA SHEET

LANCE® Ultra Research Reagents

LANCE *Ultra* EGF and EGFR (Human) Binding Kit

Product number: TRF1366 C/M

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

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Product Information

Application: This kit is designed for the detection of binding activity between Human EGF and EGFR using a homogeneous LANCE *Ultra* assay (no wash steps). This assay can facilitate the design and development of therapeutics which competitively inhibit EGF/EGFR interaction.

Sensitivity: $K_{d(app):}$ 0.25 nM (average) using 3 nM EGFR

Signal to background ratio: 10 (average) using 3 nM EGFR

Storage: The kit components must be stored at +4 ˚C in the dark.

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.

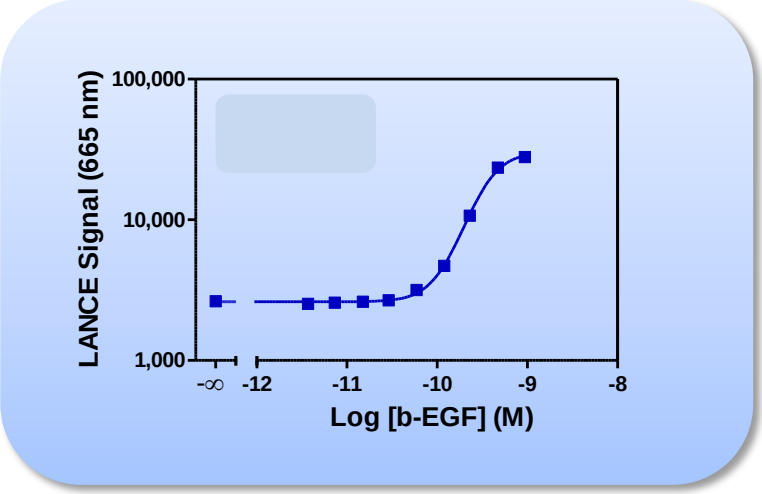


Figure 1. A typical binding curve between biotinylated EGF (bEGF) and EGFR (3 nM) performed in *Ultra* HiBlock Buffer. The data was generated using a white Optiplate™-384 microplate and the VICTOR X, ViewLux, EnVision or EnSpire Multilabel Plate Reader equipped with TR-FRET option.

Quality Control

Lot to lot consistency is confirmed in an LANCE Ultra assay. K_d and Signal/Noise were measured on the VICTOR X, ViewLux, EnVision or EnSpire Multilabel Plate Reader equipped with TR-FRET option using the protocol quality control protocol. We certify that these results meet our quality release criteria. Maximum counts may vary between lots and the instrument used, with no impact on K_d measurement.

Analyte of Interest

Epidermal Growth Factor Receptor (EGFR, Her1, ErbB1) is a 134 kDa cell surface receptor, part of a four member subfamily of receptor tyrosine kinases (erbB1, erbB2, erbB3, and erbB4). EGFR binds to a family of proteins called the epidermal growth factors (primarily EGF), which, upon association induces dimerization of the receptor and initiates signal transduction for the promotion of cell survival and growth. Mutations that cause aberrant overexpression of EGF and/or EGFR have been linked with many different cancers such as non-small-cell lung and colon cancers. Drugs are being developed as a means to better treat these types cancer.

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. Streptavidin is labeled with a donor fluorophore (a LANCE Europium chelate) which binds to biotinylated EGF and the EGFR-Fc is recognized by anti-human IgG 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

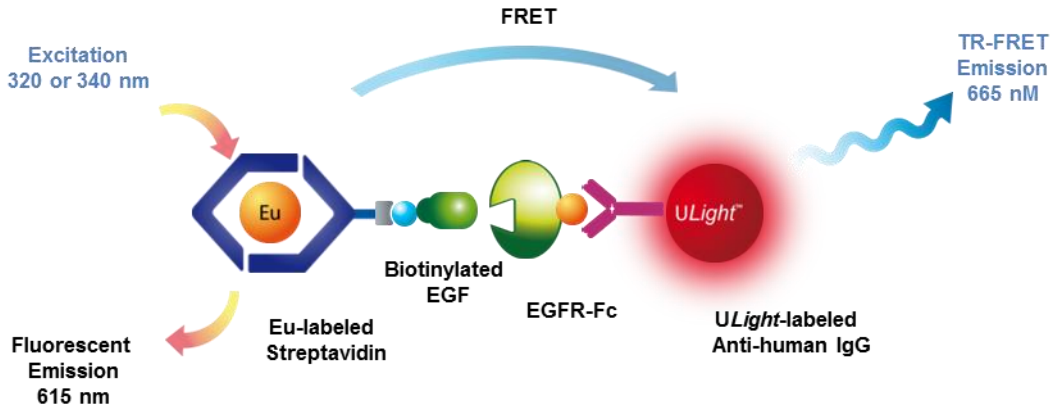


Figure 2. LANCE *Ultra* Assay Principle.

Precautions

- All blood components and biological materials should be handled as potentially hazardous.
- Some analytes are present in saliva. Take precautionary measures to avoid contamination of the reagent solutions.

Kit Content: Reagents and Materials

Kit components	TRF1366C (500 assay points)	TRF1366M (10 000 assay points)
LANCE Eu-W1024 labeled Streptavidin stored in TSA buffer, 0.1%BSA	10 µL @ 10 µg/mL (1 clear tube, yellow cap)	200 µL @ 10 µg/mL (1 clear tube, orange cap)
LANCE *Ultra* U*Light*-labeled Anti-hIgG stored in TSA buffer, 0.1% BSA	100 µL @ 500 nM (1 brown tube, blue cap)	2 X 1 mL @ 500 nM (2 brown tubes, green caps)
Human EGF (Biotinylated) lyophilized	0.3 µg (1 tube, clear cap)	0.3 µg (8 tubes, clear cap)
Human EGFR -Fc lyophilized	9.56 µg (1 tube, clear cap)	9.56 µg (6 tubes, clear cap)
Ultra HiBlock Buffer (5X)	2 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute biotinylated EGF and EGFR in 100 and 200 µL Milli-Q® grade H_2O respectively. The reconstituted proteins should be used within 60 minutes or aliquoted into screw-capped polypropylene vials and stored at -20°C for further experiments. Avoid multiple freeze-thaw cycles.

** Extra buffer can be ordered separately (cat # TRF1011C: 10 mL, cat # TRF1011F: 100 mL). 5X Ultra HiBlock Buffer may appear cloudy, especially after storage at cold temperature. Agitate and/or stir at room temperature to redissolve prior to dilution.

*** The number of assay points is based on an assay volume of 50 µL in 96-well plates, 20 µL in 384-well Optiplates or 20 µL in 384 well ProxiPlates 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 LANCE *Ultra* 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
VICTOR X, ViewLux, EnVision or EnSpire Multilabel Plate Reader equipped with TR-FRET option	PerkinElmer Inc.	-

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 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 ULight 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.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment. The standard curve should be performed in Ultra HiBlock Buffer

Competition Assay Procedure

			Volume				
Format	**# of data points**	**Final**	**Inhibitor Or Antibody**	**EGFR**	**Biotinylated EGF**	**U*Light* anti-IgG + Eu-SA**	**Plate recommendation**
TRF1366 C	250	40 µL	10 µL	10 µL	10 µL	10 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	20 µL	5 µL	5 µL	5 µL	5 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290)
	1 250	8 µL	2 µL	2 µL	2 µL	2 µL	ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	4 µL	1 µL	1 µL	1 µL	1 µL	White OptiPlate-1536 (cat # 6004290)
TRF1366 M	5000	20 µL	10 µL	10 µL	10 µL	10 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290)
	12 500	8 µL	2 µL	2 µL	2 µL	2 µL	ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	4 µL	1 µL	1 µL	1 µL	1 µL	White OptiPlate-1536 (cat # 6004290)

Example: Competition using target and non-target antibodies

- The protocol described below is an ***example*** for generating 500 wells, triplicate determinations, including three inhibition curves in a 20 µL final assay volume. If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.

- The dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.

The following reagents were used for the application example shown below:

Item (Ab)	Supplier	Catalog number
Anti-human EGFR antibody	R & D Systems	AF231

1 step protocol (1 Incubation steps) described as below:

1) Preparation of 1X *Ultra* HiBlock Buffer:
   Add 2 mL of 5X *Ultra* HiBlock Buffer to 8 mL H_2O.

2) Preparation of serial dilutions of 4X anti-human EGFR antibody in 1X *Ultra* HiBlock Buffer as follows:

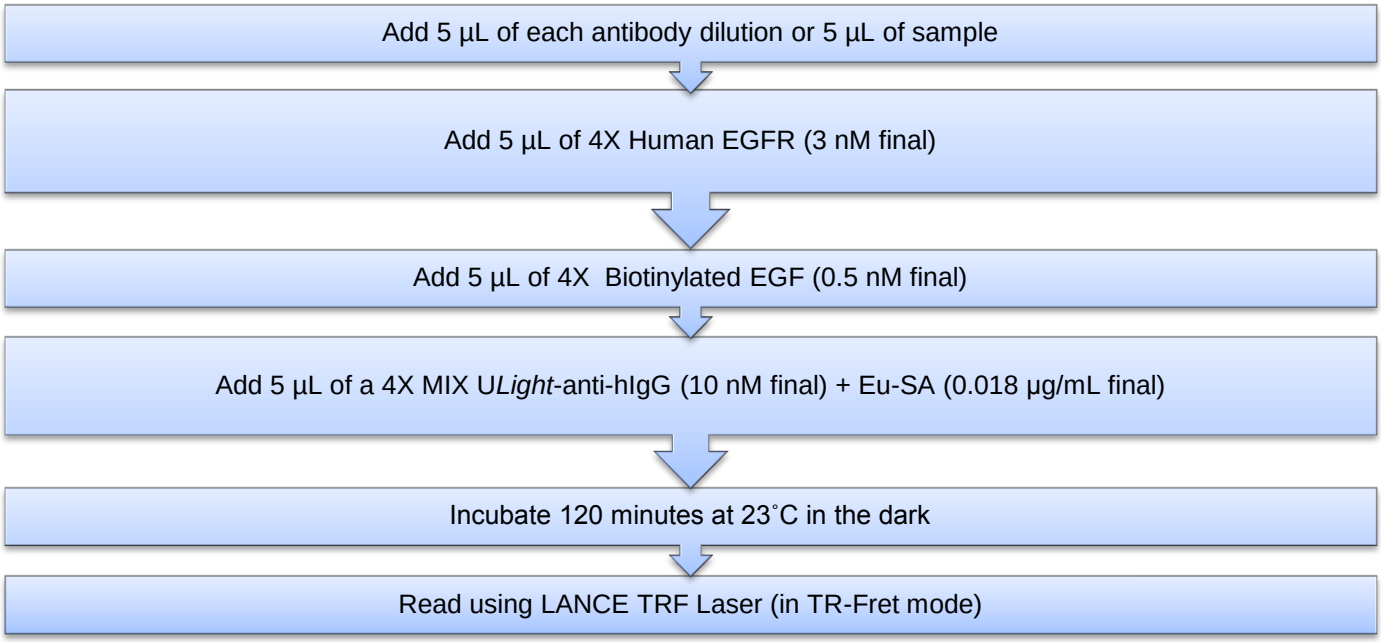
Tube	Volume of Antibody	Volume of 1X buffer	[Ab] (g/mL) (1X) final concentration
A	Add 30 µL of 1E-3 g/mL stock	45	1.00E-04
B	30 µL of tube A	70	3.00E-05
C	30 µL of tube B	60	1.00E-05
D	30 µL of tube C	70	3.00E-06
E	30 µL of tube D	60	1.00E-06
F	30 µL of tube E	70	3.00E-07
G	30 µL of tube F	60	1.00E-07
H	30 µL of tube G	70	3.00E-08
I	30 µL of tube H	60	1.00E-08
J	30 µL of tube I	70	3.00E-09
K	30 µL of tube J	60	1.00E-09
L	30 µL of tube K	70	3.00E-10
M-Max Signal	0	100 µL	0
N-Max Signal	0	100 µL	0
O-Max Signal	0	100 µL	0
P-Max Signal	0	100 µL	0

3) Preparation of 4X Mix of U*Light* labeled Anti-Human IgG Fc-specific Antibody (40 nM) and Eu-W1024 labeled-Streptavidin (1.2 nM):
   a. Add 18 µL of Eu-W1024 Streptavidin at 10 µg/mL and 200 µL of U*Light* anti Human IgG at 500 nM to 2282 µL of 1X *Ultra* HiBlock Buffer
   b. Prepare just before use.

4) Preparation of 4X biotinylated EGF(2 nM):
   a. Prepare just before use.
   b. Reconstitute lyophilized biotinylated EGF (0.3 µg) in 100 µL H_2O to make 500 nM
   c. Add 10 µL of 500 nM biotinylated EGF to 2490 µL 1X *Ultra* HiBlock Buffer.

5) Preparation of 4X EGFR Fc-tagged (12 nM):
   a. Reconstitute lyophilized EGFR Fc-tagged (9.56 µg) in 200 µL H_2O to make 500 nM stock concentration.
   b. Add 60 µL of 500 nM EGFR Fc-tagged to 2440 µL of 1X *Ultra* HiBlock Buffer.
   c. Prepare just before use.

6) In a white Optiplate (384 wells):



Important: LANCE signal is detected using an EnVision 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).

Sample Results:

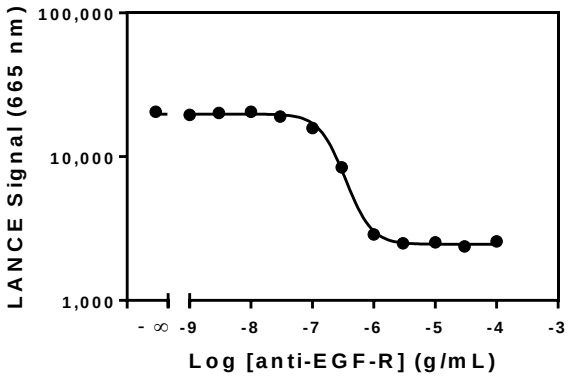


Figure 3. Antibody Inhibition curve using EGFR antibody. Anti-Human EGFR (calculated IC_{50} of 215 nM)

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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