TECHNICAL DATA SHEET

LANCE® *Ultra* Research Reagents

# LANCE *Ultra* FCGR3A/CD16a (176Phe/F158) Kit

Product number: TRF1347 C/M

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

## Contents

## ○ Product Information

| | |
|---|---|
| **Application:** | This kit is designed for the quantitative determination of Human FCGR3A/CD16a (176Phe/F158) and human Fc fragment binding using a homogeneous LANCE *Ultra* assay (no wash steps). |
| **Sensitivity:** | $EC_{50}$: 0.5 nM |
| | Signal / Background ratio: 13 |
| **Storage:** | Store kit in the dark at +4˚C. Store reconstituted analyte at -20°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 an LANCE *Ultra* assay. $EC_{50}$ and LDL were measured on the VICTOR *X*, ViewLux, EnVision or EnSpire Multilabel Plate Reader equipped with TR-FRET option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between lots and the instrument used, with no impact on LDL measurement.

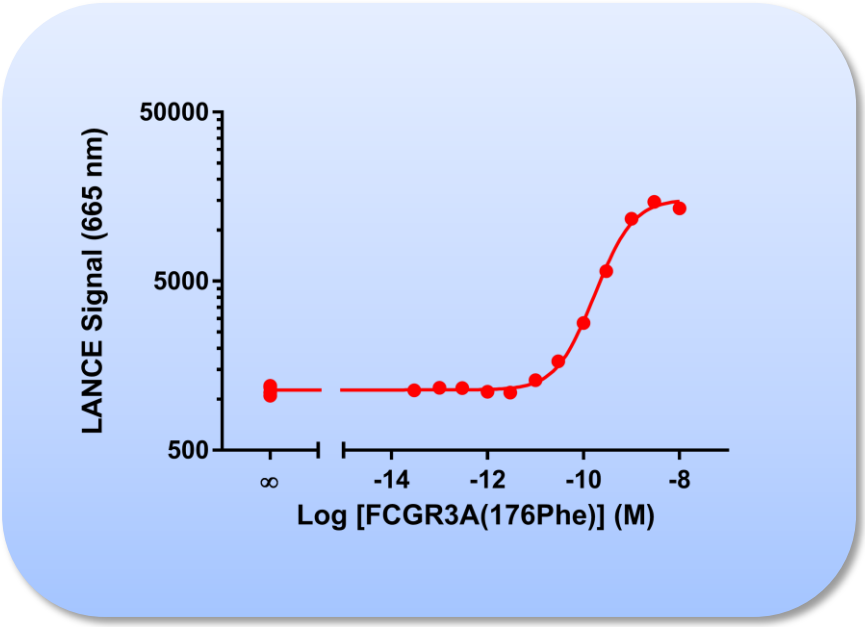



**Figure 1**. Typical sensitivity curves in *Ultra* HiBlock Buffer. The data was generated using a white Optiplate™-384 microplate and the VICTOR *X*, ViewLux, EnVision or EnSpire Multilabel Plate Reader equipped with TR-FRET option

## Analyte of Interest

The Fc-Gamma Receptors (FCGRs) are members of immunoglobulin superfamily and play a critical role in the function of therapeutic antibodies. FCGRs are divided into three classes and FCGR3 (CD16) is expressed as two distinct forms (FCGR3A and FCGR3B) encoded by two different highly homologous genes in a cell type specific manner. FCGR3A is a low/intermediate affinity receptor for polyvalent immune-complexed IgG. It is involved in phagocytosis, secretion of enzymes and inflammatory mediators, antibody-dependent cellular cytotoxicity (ADCC), mast cell degranulation and clearance of immune complexes. In humans, a single nucleotide polymorphism creates two isoforms: high binding (176Val/V158) and low binding (176Phe/F158) forms that, when homozygous, may influence susceptibility to autoimmune diseases or response to therapeutic IgG antibodies. FCGR3A has been considered as an important therapeutic target. This LANCE *Ultra* assay can be used to determine the binding activity of human IgG Fc fragment to human FCGR3A and also can be used to study how other antibodies bind to FCGR3A by competition assay.

## 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 protein of interest is labeled with a donor fluorophore (a LANCE Europium chelate) and the second protein 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.

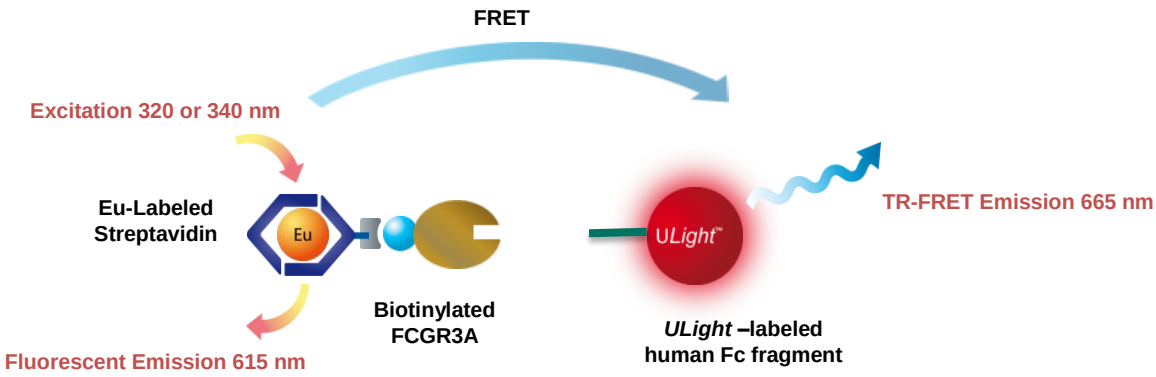



**Figure 2**. LANCE assay principle.

## 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 | TRF1347C (500 assay points) | TRF1347M (10 000 assay points) |
|---|---|---|
| LANCE *Ultra* Eu-labeled Streptavidin | 25 µL @ 10 µg/mL (1 clear tube, yellow cap) | 500 µL @ 10 µg/mL (1 clear tube, orange cap) |
| LANCE *Ultra* U*Light*-labeled human Fc fragment | 120 µL @ 5 µM (1 brown tube, blue cap) | 3 x 1 mL @ 5 µM (3 brown tubes, green caps) |
| Biotinylated human FCGR3A/CD16a(176Phe/F158) Lyophilized | 5 µg (1 tube, clear cap) | 5 µg (1 tube, clear cap) |
| *Ultra* HiBlock Buffer (5X) | 2 mL, 1 small bottle | 100 mL, 1 large bottle |

* Reconstitute biotinylated FCGR3A(176Phe) in 100 µL Milli-Q® grade $H_2O$. The reconstituted protein 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 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 # |
|---|---|---|
| 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. | - |

The following reagents might be required for particular applications:

| Item | Supplier | Catalog number |
|---|---|---|
| IgG1 Human Plasma | Athens Research Technology | 16-16-090707-1 |
| IgG2 Human Plasma | Athens Research Technology | 16-16-090707-2 |
| IgG3 Human Plasma | Athens Research Technology | 16-16-090707-3 |
| IgG4 Human Plasma | Athens Research Technology | 16-16-090707-4 |
| ChromPure Human IgG F(ab')$_2$ Fragment | JacksonImmunoResearch | 009-000-006 |
| ChromPure Human IgG Fc Fragment | JacksonImmunoResearch | 009-000-008 |
| ChromPure Human IgG,whole molecule | JacksonImmunoResearch | 009-000-003 |
| Anti-human CD16a antibody | AbD Serotec | MCA1193GA |

## ○ 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.
- 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. Centrifuge the assay plate (x1000 rpm, 30 sec) is recommended.
- 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.

## Competition Assay Protocol

- Assay specificity can be demonstrated by competing the binding of human FCGR3A with all subclasses of human IgG or comparing the binding with human IgG fragments.

The competition assay described below is an example for determining $IC_{50}$ of human IgG subclass competitive binding to human FCGR3A in 20 µL final assay volume (384 wells, duplicate determinations) by LANCE *Ultra* technology. This protocol can test 500 sample points. If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly. The protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.

1. Preparation of 1X LANCE *Ultra* HiBlock Buffer (for 10 mL)
   Add 2 mL of 5X LANCE *Ultra* HiBlock Buffer and 8 mL of ultrapure water (18 MΩ.cm).

2. Preparation of serial dilution of human IgG subclasses
   Prepare serial dilutions of 4X IgG in 1X LANCE *Ultra* HiBlock buffer as follows:

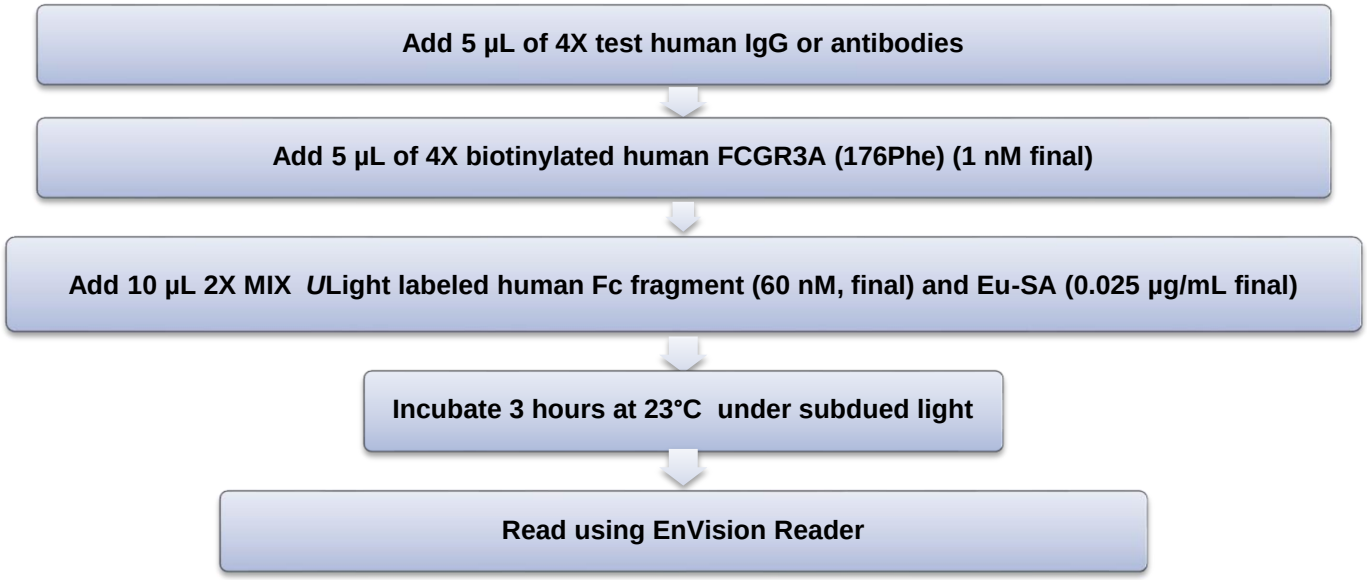
| Tube | Volume of IgG | Volume of 1X buffer | [IgG] (M) (4X) | [IgG] (M) (1X) |
|---|---|---|---|---|
| A | 1.2 mM stock | 0 | 1.20E-03 | 3.00E-04 |
| B | 30 µL of tube A | 60 µL | 4.00E-04 | 1.00E-04 |
| C | 30 µL of tube B | 70 µL | 1.20E-04 | 3.00E-05 |
| D | 30 µL of tube C | 60 µL | 4.00E-05 | 1.00E-05 |
| E | 30 µL of tube D | 70 µL | 1.20E-05 | 3.00E-06 |
| F | 30 µL of tube E | 60 µL | 4.00E-06 | 1.00E-06 |
| G | 30 µL of tube F | 70 µL | 1.20E-06 | 3.00E-07 |
| H | 30 µL of tube G | 60 µL | 4.00E-07 | 1.00E-07 |
| I | 30 µL of tube H | 70 µL | 1.20E-07 | 3.00E-08 |
| J | 30 µL of tube I | 60 µL | 4.00E-08 | 1.00E-08 |
| K | 30 µL of tube J | 70 µL | 1.20E-08 | 3.00E-09 |
| L | 30 µL of tube K | 60 µL | 4.00E-09 | 1.00E-09 |

3. Preparation of 4X biotinylated human FCGR3A (176Phe) (4 nM, 3000 µL)
   a. Spin the vial containing 5 µg lyophilized protein briefly in microfuge and reconstitute it with 100 µL sterile distilled water to make 2 µM stock concentration of human FCGR3A (176Phe).
   b. Add the 6 µL of 2 µM human FCGR3A (176Phe) into a new tube containing 2994 µL 1X *Ultra* HiBlock buffer to make 4 nM human FCGR3A (176Phel).
   c. Prepare just before use.

4. Preparation 2X MIX U*Light* labeled human Fc fragment and Eu labeled Streptavidin (Eu-SA) (120 nM / 0.05 µg/mL, 5000 µL)

a. Add 120 µL of 5 µM U*Light* labeled human Fc fragment and 25 µL of 10 µg/mL Eu-SA into 4855 µL 1X *Ultra* HiBlock buffer
b. Prepare just before use.

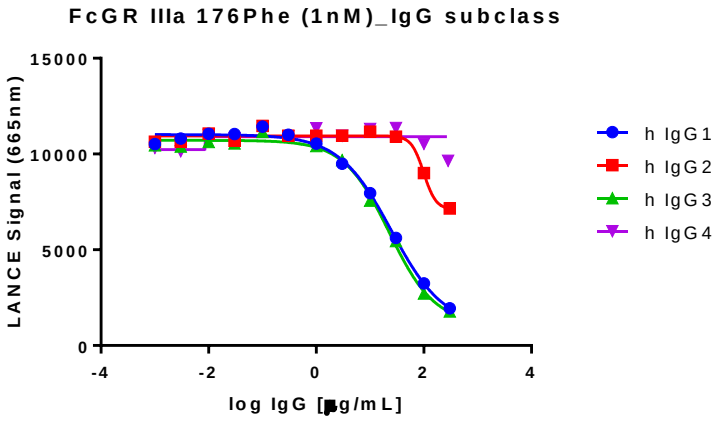
5. In a white Optiplate (384 wells):




**Important:**

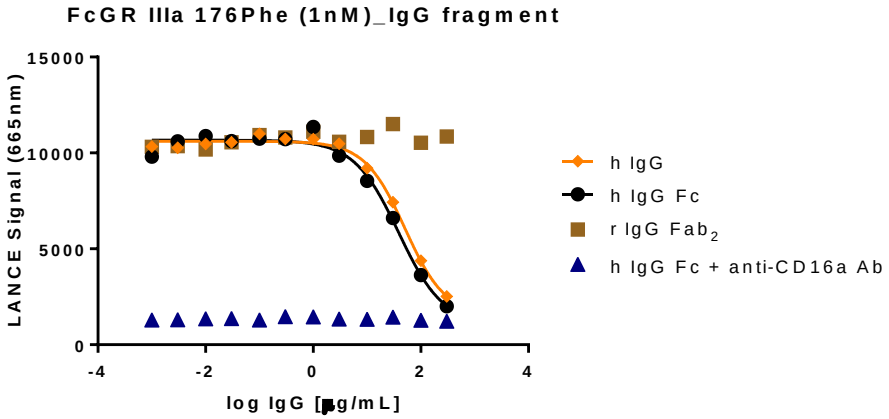
- LANCE signal is detected using an EnVision Multilabel Reader equipped with the TR-FRET. Use an excitation wavelength of 320 or 340 nm to excite the LANCE Europium chelate. We recommend you read this assay in dual emission mode, detecting both the emission from the Europium donor fluorophore at 615 nm, and the acceptor fluorophore (at 665 nm for U*Light* dye). The raw FRET signal at 665 nm or FRET signal ratio (665nm/615nm) can be used to process your data.
- Assay results are stable for up to 24 hours

**Typical competitive binding Data:**




| | h IgG1 | h IgG2 | h IgG3 |
|---|---|---|---|
| IC50 | 25.07 | 99.41 | 22.65 |

**Figure 3**. Human IgG subclasses competitive bind to FCGR3A (176Phe) (1 nM). The $IC_{50}$ values are 25.1, 99.4 and 22.7 µg/mL for IgG1, IgG2 and IgG3 respectively and were calculated by using nonlinear regression fitting with GraphPad Prism 5.




| | h IgG | h IgG Fc |
|---|---|---|
| IC50 | 50.85 | 38.82 |

**Figure 4.** Human IgG fragments competitive bind to FCGR3A (176Phe) (1 nM). Blue triangle points showed human IgG Fc fragment competed binding to blocked FCGR3A(176Val) which was pre-incubated with anti-human CD16a antibody (10 µg/mL) for 5 minutes at room temperature as a negative control. The IgG Fab fragment acts as a positive control. The $IC_{50}$ values are 50.9 and 38.8 µg/mL for IgG whole molecule and IgG Fc fragment respectively and were calculated by using nonlinear regression fitting with GraphPad Prism 5.

## ○ Troubleshooting Guide

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

www.perkinelmer.com/lancetroubleshoot

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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



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**