DiscoverX

User Manual
KILR® Detection Kit

Chemiluminescent Assay for Immune-Mediated Cytotoxicity

Please read this entire user manual before proceeding with the assay.
For additional information or Technical Support, see contact information below.

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Overview

The KILR Detection Kit is to be used with the KILR Assays for Adherent or Suspension Cell Lines when running ADCC or other immune assays that result in cell lysis. The kit is easy-to-use and has been successfully run in 96-well microplate format. The resulting signal is chemiluminescent and is read with any standard plate reader.

KILR Assay products utilize the Enzyme Fragment Complementation (EFC) technology for studying cell cytotoxicity with the aid of the complementation of two β-galactosidase (β-gal) fragments. The larger β-gal fragment is called Enzyme Acceptor (EA) and the smaller fragment is termed Enzyme Donor (ED; also known as enhanced ProLabel® [ePL]). The two fragments are inactive when apart. However, when they complement, they form a functional enzyme that hydrolyzes the substrate to generate a chemiluminescent signal. The detection reagent contains the EA fragment of β-gal, and therefore must be used in conjunction with KILR cell lines, which express the ePL-tagged KILR Reporter Protein in order to obtain a detectable signal.

Protocols for cytotoxicity applications other than ADCC may require a different DiscoverX assay detection kit. The mechanism of cell death will dictate which detection reagent will be needed for developing an assay. Cytotoxic treatments that cause a loss of plasma membrane integrity that results in cell lysis (similar to ADCC mediated apoptosis), therefore releasing the KILR Reporter Protein into the assay media, require the use of this KILR Detection Kit (Cat. No. 97-0001 Series). However, cytotoxicity assays that kill cells without compromising the integrity of the plasma membrane (e.g. ADCP assays) will require the PathHunter® PL/PK Detection Kit (Cat. No. 93-0812 Series) which contains a cell lysis buffer to artificially lyse the cells.

Materials Provided

Catalog Number	**97-0001S**	**97-0001M**	**97-0001L**
Sufficient for (Number of Plates)*	2	10	25
96-Well Plate (Data Points)	~200	~1,000	~2,500
Kit Components	**Volume in Each Bottle**		
KILR Detection Reagent 1 (mL)	17	85	212.5
KILR Detection Reagent 2 (mL)	5	25	62.5
KILR Detection Reagent 3 (mL)	5	25	62.5
KILR Total Lysis Control (mL)**	0.5	2.5	6.25

*Assay plates not provided with kit.
** There may be excess reagent remaining in the bottle, after using recommended volumes in the assay.

Storage Conditions

Upon arrival, store reagents at -20°C. The detection kit is stable until the expiry date indicated on the kit box outer label. Thaw frozen detection reagents at room temperature before use. After thawing, store detection reagents for up to 1 month at 2-8°C. For longer term storage, aliquots of all the detection components may be re-frozen in opaque containers at -20°C. The detection reagents can be thawed and frozen for a total of 3 times without loss in performance.

Additional Materials Required

Material	Ordering Information
KILR Assay Cell Line	Refer to cell line-specific datasheet
Disposable Reagent Reservoir	Thermo Fisher Scientific, Cat. No. 8094 or similar
15 mL Polypropylene Tubes	
Multimode or Luminescence Reader	discoverx.com/instrument-compatibility

Assay Detection Protocol

Upon completion of opsonization and effector cell treatment steps, the KILR assay is ready for the detection step. The following section contains procedures for adding the Total Lysis Control Reagent (to selected control wells) and/or the KILR Detection Reagent, and reading the assay plate on a luminometer.

1. Prepare working KILR detection solution in a 15 mL tube or a reagent reservoir by mixing 4-parts of KILR Detection Reagent 1, 1-part of KILR Detection Reagent 2, and 1-part of KILR Detection Reagent 3 (including excess volume for accurate pipetting). Mix reagents by gently inverting the tube twice or swirling the reagent reservoir.

Working KILR Detection Solution		
Detection Components	**Volume Ratio**	**Volume Per Plate (mL)**
KILR Detection Reagent 1	4	8
KILR Detection Reagent 2	1	2
KILR Detection Reagent 3	1	2
Total Volume		**12**

Working KILR detection solution is light sensitive, thus incubation in the dark is necessary.

Spontaneous Control and Total Lysis Control are described in the KILR Assays for Cell Lines user manuals.

2. Add 2 µL per well of the KILR Total Lysis Control Reagent ONLY to the wells set aside for the Total Lysis Control. Do not add the reagent to the wells treated with the test antibodies or to the Spontaneous Release Control wells.

We recommend adding Total Lysis Control reagent to the Total Lysis Control wells at the same time as effector cells are added to the sample wells, especially for assays requiring long incubation times (>3 hours).

3. Add 100 µL of working KILR detection solution Solution to the wells in the assay plate containing the KILR target cells, including the Total Lysis and Spontaneous Release Control wells. It is not recommended to mix wells by pipetting up and down or by vortexing the assay plate.

4. Incubate the assay plate for at least 1 hour at room temperature in the dark.

5. Read samples on a standard luminescence plate reader at 0.1 to 1 second/well for photomultiplier tube readers or 5 to 10 seconds for imager. The actual signal characteristics over time are affected by lab conditions, such as temperature, and the user should establish an optimal read time. In general, the signal continues to increase. The plate may be incubated overnight (16 hours) and the signal may be measured the next day. Once an optimal read time has been established, continue to use this incubation time to maintain consistency between assays. Luminescence readout usually collects signal from all wavelengths. Some instrument manufacturers may include a cutoff filter at high wavelengths, but usually no wavelength setting is needed for luminescence readout.

6. Data analysis can be performed using your choice of statistical analysis software (e.g. GraphPad Prism, Molecular Devices Softmax Pro, BioTek Gen5, Microsoft Excel, etc.).

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