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Assessing IL-15 bioavailability ("the bioassay")

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ABSTRACT

Assessing the capacity of IL-15 analogs (e.g. within TriKE molecules) to stimulate proliferation of NK-92 cells. NK-92 are deprived of cytokines overnight, then cultured with IL-15 analogs for two days. After this time, the expansion of viable cells is quantitatively assessed using a redox-sensitive dye that changes from blue/non-fluorescent in media to pink/fluorescent upon reduction in viable cells.

It should be noted that NK-92 lack CD16, so delivery of IL-15 to these cells should not be enhanced by the anti-CD16 component of the TriKE as it would be for CD16+ NK-92 or CD16+ pNK cells.

This protocol is adapted from the following assays:
https://linkinghub.elsevier.com/retrieve/pii/0022175994903964

http://doi.wiley.com/10.1046/j.1440-1711.1998.00733.x

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MATERIALS TEXT

MATERIALS

96 well black assay plate with clear bottom **Corning Catalog #3603**

Resazurin **R&D Systems Catalog #AR002** Step 6

NK-92 **ATCC Catalog #CRL-247** Step 1

NK-92 culture

1 Defrost NK-92 (malignant non-Hodgkin's lymphoma; see *Cell Line Information*) and culture for at least one week prior to initiation of the assay.

NK-92 **ATCC Catalog #CRL-247**

1.1 NK-92 media

- Alpha Minimum Essential Medium plus ribonucleosides and deoxyribonucleosides (Gibco Cat. No. 12571)
- 0.1 mM 2-mercaptoethanol (Sigma Aldrich, Cat. No. M7522)
- 12.5% horse serum (Fisher Scientific Cat. No. 26050088)
- 12.5% fetal bovine serum (FBS; Gibco Cat. No. 26140079)
- 100 U/mL penicillin streptomycin (Gibco Cat. No. 15140122)

For normal culture, supplement with 500 U/mL recombinant human IL-2 (Prometheus, Cat. No. NDC 65483-116-07).

2-Mercaptoethanol **Sigma Aldrich Catalog #M7522**

Horse Serum, heat inactivated, New Zealand origin **Thermo Fisher Catalog #26050088**

Fetal Bovine Serum, qualified, United States **Thermo Fisher Catalog #26140079**

Penicillin-Streptomycin **Gibco - Thermo Fisher Catalog #15140122**

Proleukin (recombinant IL-2) **Contributed by users Catalog #National Drug Code 65483-116-07**

1.2 NK-92 culture conditions:
Humidified incubator at 37.0°C, 5% CO_2

Culture conditions: Replace medium every 2 - 3 days
Passage interval: Passage 2 - 3 times a week
Sub-cultivation ratio: 1:2 - 1:3

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Seeding conditions: $2x10^5$ - $3x10^5$ cells/mL

1.3 NK-92 freezing protocol:
- Freeze medium: 50% FBS; 40% NK92 media; 10% DMSO
- Freeze no more than $5x10^6$ cells/mL
- Storage temperature: liquid nitrogen vapor phase

Day - 1

2 Deprive NK-92 of IL-2 at least 16 h prior to plating cells.

2.1 Count NK cells (at least 4.8 million will be required for a full plate).

2.2 Spin NK-92 at 1600 RPM for 5 min and remove the supernatant.

2.3 Resuspend cells in media without IL-2.

2.4 Spin NK-92 at 1600 RPM for 5 min and remove supernatant.

2.5 Resuspend cells at 7.5 x 10^5cells/mL in media without IL-2 and return to the incubator overnight.

Day 0

3 Dilute all drugs in media (without IL-2) to 5x the highest concentration to be tested (e.g. 150 nM). This will be used to make a serial dilution of the drug (3x, 5x or 10x depending on range required).

4 Plate NK-92 in triplicate into a 96 well black assay plate at 5 x 10^4 cells/well in 80 µL of media without IL-2. Avoid using the wells at the edge of the plate at these undergo the greatest evaporation.

5 Add 20 µL of diluted drug to each well containing NK-92. Include a no drug condition as a negative control.

Day 2

6 After 24 h, add 10 µL resazurin to each well, mix and incubate for 1 h at 37°C and 5% CO2.
Resazurin **R&D Systems Catalog #AR002**

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7 Ensuring there are no air bubbles in the media, read the fluorescence at 530-570 nm, with a correction at 590-620 nm using a plate reader (Tecan).

8 Replace the plate in the incubator and wait 1 h before repeating step 7. Repeat this process to obtain four readings in total. Select the most stable readings for analysis (usually 3 h).

9 Analyze the adjusted readings using Graphpad Prism. Log transform the data and calculate a non-linear fit using "log(agonist) vs. normalized response -- Variable slope - Least squares fit" in order to obtain an EC 50.

Prism 8.0
by GraphPad

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