

Enable-NK™ Duo 10X: Instructions for Use

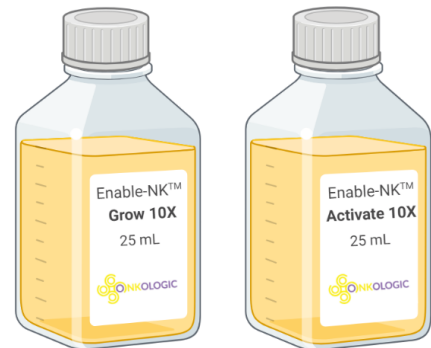
- ❖ **This document provides simple guidelines for optimal use of the product; please refer to the “Suggested Protocols” document for more detailed cell culture methodology information.**

Storage: 2 - 8 °C. Please note that it is OK for the shipped product to arrive at room temperature. Do not use if the product packaging is damaged. Do not use if labeling is incomplete or illegible.

Product Description

Enable-NK™ Duo 10X is a set of 2 cell culture media concentrates consisting of Enable-NK™ Grow 10X and Enable-NK™ Activate 10X, formulated specifically for human natural killer (NK) cells, and is xeno-free. These formulations are recommended for primary NK cells as well as NK cell lines. These 10X formulations can be added as supplements to any cell culture media, in order to create custom media formulations for your needs.

- Enable-NK™ Grow 10X is a cell culture supplement (as a 10X concentrate) for the expansion (proliferation) of NK cells. Upon 10-fold dilution with a cell culture medium of choice, it also provides the option of gradual reduction in serum content during cellular expansion. It is xeno-free, and is available devoid of animal-derived products while being chemically defined. Plant extracts (which are part of the Enable-NK™ Duo formulation) are present in Enable-NK™ Activate, but not in Enable-NK™ Grow. Enable-NK™ Grow is formulated with a unique combination of amino acids, fatty acids, vitamins, and other ingredients.
- Enable-NK™ Activate 10X is a cell culture supplement (as a 10X concentrate) for increasing the cytotoxic activity of cultured human NK cells toward target cancer cells, while also continuing to support cell proliferation. It is xeno-free, and is available devoid of animal-derived products. Enable-NK™ Activate is formulated with all the ingredients of Enable-NK™ Grow and a unique combination of additional ingredients. These additional ingredients include United States Pharmacopeia (USP) compendial-grade plant extracts.



Most media products developed for NK cell culture are designed for *simultaneous* expansion and activation. As a result, due to the extended period of activation, the expanded NK cells often express inhibitory receptors (i.e. exhaustion markers) and display reduced functionality. Enable-NK™ Duo 10X, deliberately comprising two distinct formulations, is specifically designed for separating the expansion phase from the activation phase, thus reducing the probability of NK cell exhaustion.

While it is true that upon 10-fold dilution, *both* media supplements support NK cell expansion as well as activation, the best results will be achieved with the use of Enable-NK™ Grow 10X *prior* to the use of Enable-NK™ Activate 10X. Please see the detailed instructions for use in the next few pages.

Enable-NK™ Duo 10X: Instructions for Use

Instructions for the 10-fold dilution of Enable-NK™ Duo 10X supplements into media:

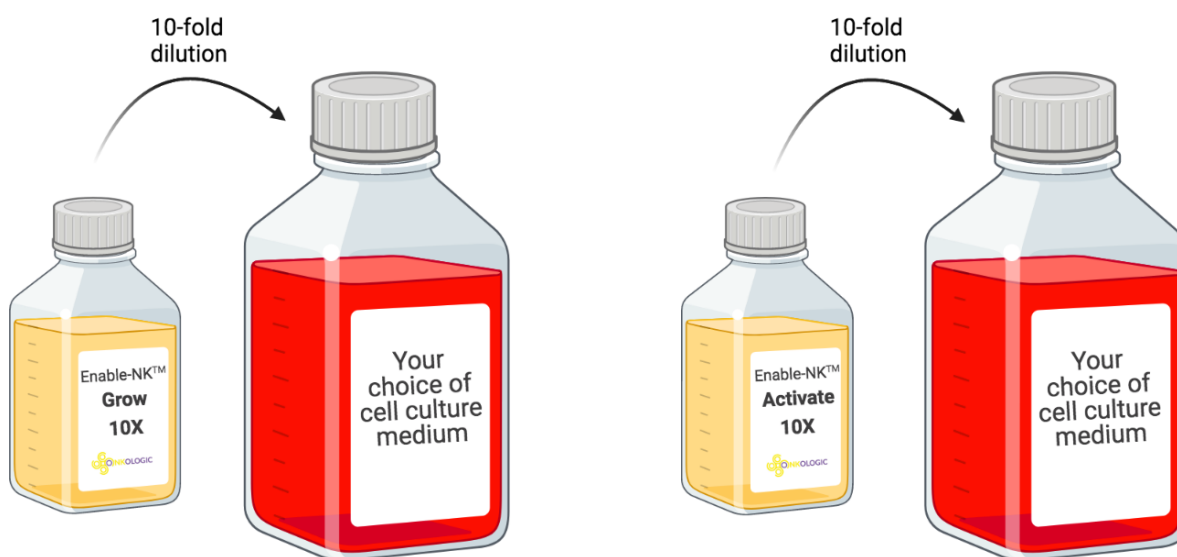


Figure legend: Schematic demonstrating the addition of Enable-NK™ 10X products into a culture medium of choice.

Add Enable-NK™ Grow 10X to cell culture medium:

- Dilute the 10X supplement into your culture medium of choice by a factor of 10.
- For example, add 25 mL of the 10X supplement to 225 mL for a total of 250 mL.

Add Enable-NK™ Activate 10X to cell culture medium:

- Dilute the 10X supplement into your culture medium of choice by a factor of 10.
- For example, add 25 mL of the 10X supplement to 225 mL for a total of 250 mL.

Once the two dilutions have been completed, you will have two separate formulations:

- custom Enable-NK™ Grow medium, and
- custom Enable-NK™ Activate medium.

Further instructions for the complete preparation of these two formulations are described below.

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Instructions for the optimal preparation and use of custom Enable-NK™ Grow, after preparation (i.e. 10-fold dilution) described above:

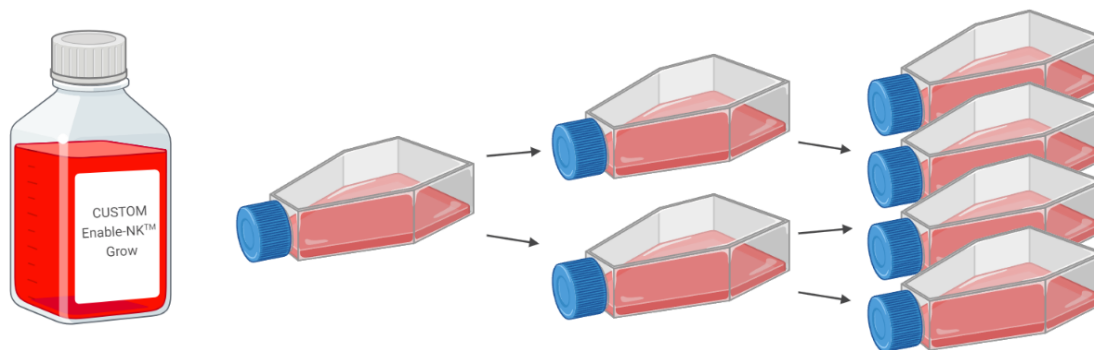


Figure legend: Enable-NK™ Grow has been optimized for expansion of NK cells, for e.g. by splitting using T-flasks.

Additives (if no serum reduction is desired; additives are not included with product):

- For NK cell lines:
 - 10–20% fetal bovine serum (FBS)
 - * Recommended: 20% FBS
 - 50–250 U/mL of IL-2
 - * Recommended: 100 U/mL IL-2
 - 1% penicillin/streptomycin
 - Additional additives for **Enable-NK™ Grow 10X Protein-free product version ONLY:**
 - Human serum albumin (HSA), Sigma catalog # A9731
 - Reconstitute and add to achieve a final concentration of 100 µg/mL.
 - Insulin/transferrin/selenite (ITS), Corning catalog # 25-800-CR
 - Add to achieve a 50-fold dilution of ITS into the medium.
- For primary NK cells:
 - 5–10% pooled human AB serum (HS)
 - * Recommended: 10% HS
 - 50–250 U/mL of IL-2
 - * Recommended: 100 U/mL IL-2
 - 1% penicillin/streptomycin
 - Additional additives for **Enable-NK™ Grow 10X Protein-free product version ONLY:**
 - Human serum albumin (HSA), Sigma catalog # A9731
 - Reconstitute and add to achieve a final concentration of 100 µg/mL.
 - Insulin/transferrin/selenite (ITS), Corning catalog # 25-800-CR
 - Add to achieve a 50-fold dilution of ITS into the medium.

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If serum reduction is desired, reduce serum content gradually i.e. in a stepwise fashion:

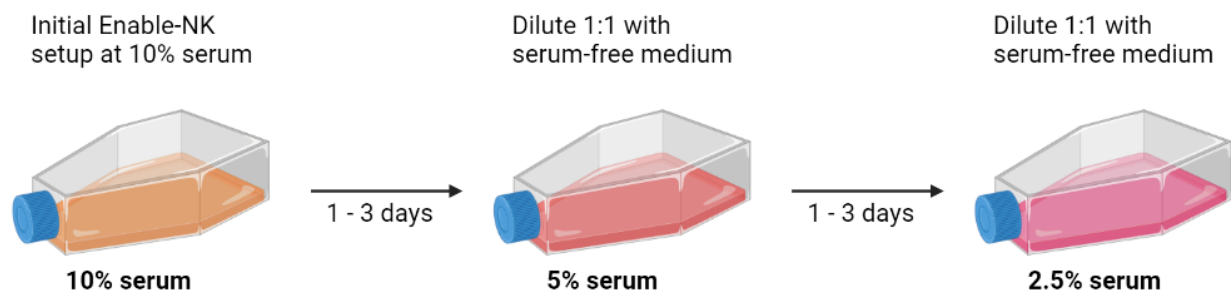


Figure legend: Schematic for suggested stepwise reduction of serum content over 3 to 7 days.

Minimal additives (following the gradual reduction in serum content illustrated above; additives are not included with product):

- For NK cell lines:
 - 2% fetal bovine serum (FBS)
 - 50–250 U/mL of IL-2
 - * Recommended: 100 U/mL IL-2
 - 1% penicillin/streptomycin
 - Additional additives for **Enable-NK™ Grow 10X Protein-free product version ONLY:**
 - Human serum albumin (HSA), Sigma catalog # A9731
 - Reconstitute and add to achieve a final concentration of 100 µg/mL.
 - Insulin/transferrin/selenite (ITS), Corning catalog # 25-800-CR
 - Add to achieve a 50-fold dilution of ITS into the medium.
- For primary NK cells:
 - 2.5% pooled human AB serum (HS)
 - 50–250 U/mL of IL-2
 - * Recommended: 100 U/mL IL-2
 - 1% penicillin/streptomycin
 - Additional additives for **Enable-NK™ Grow 10X Protein-free product version ONLY:**
 - Human serum albumin (HSA), Sigma catalog # A9731
 - Reconstitute and add to achieve a final concentration of 100 µg/mL.
 - Insulin/transferrin/selenite (ITS), Corning catalog # 25-800-CR
 - Add to achieve a 50-fold dilution of ITS into the medium.

Enable-NK™ Duo 10X: Instructions for Use

Suggestions for culture maintenance:

Cell density

- Suspension cultures (e.g. flasks) should be maintained between $0.75\text{--}2.5 \times 10^6$ cells/mL.
- Cultures using G-Rex plates:
 - Set up at no less than 0.5×10^6 cells/cm².
 - Do not allow culture to exceed 10×10^6 cells/cm².

Centrifugation parameters

- RCF range: 200–350 g * *Recommended: 300 g for 5 minutes*
- Avoid centrifugation of NK cells if possible (see Option 2 below).

When should the “Grow” phase end and the “Activate” phase begin?

- When the target cell number / fold expansion has been achieved, or cell numbers plateau
- When cells have fully adapted to desired (lower) serum concentration
- At minimum, when cells have spent at least 3 days in Enable-NK™ Grow medium

Options for transitioning cultures from Enable-NK™ Grow to Enable-NK™ Activate:

Option 1 – Full switch to Enable-NK™ Activate by centrifuging cells

- Collect all cells in centrifuge tubes, and centrifuge (*recommended 300 g for 5 minutes*).
- **Do not let cells remain pelleted for more than 30 seconds after spin is completed!**
- Resuspend pellets in Enable-NK™ Activate at desired density; distribute to new flasks.

Option 2 – Gradual switch to Enable-NK™ Activate by stepwise addition to Enable-NK™ Grow

- This method avoids centrifugation of the NK cells, which we recommend.
- Add Enable-NK™ Activate directly to the existing Enable-NK™ Grow culture.
- After counting cells, add enough Enable-NK™ Activate to achieve desired density.
- Repeat over the course of several additions (see demonstrative figure below).
- Activation effect of Enable-NK™ Activate does not require 100% Enable-NK™ Activate.

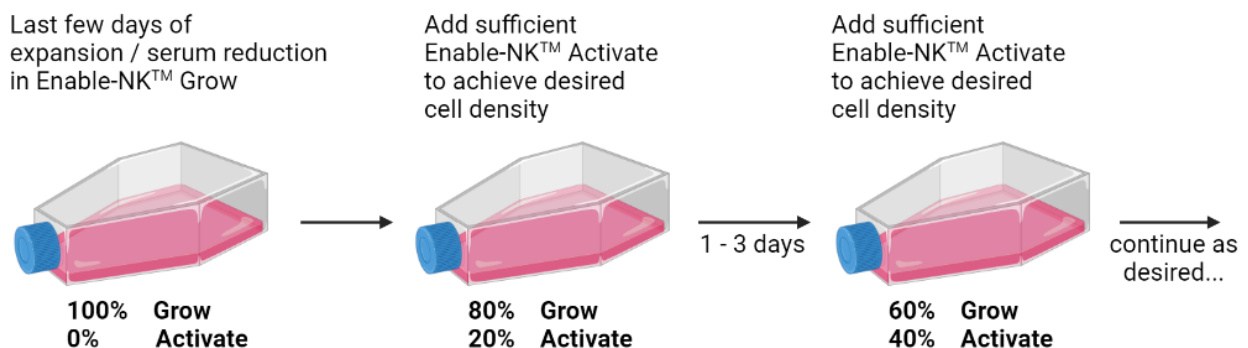


Figure legend: Schematic for gradual transition from Grow to Activate without centrifugation of cells.

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Instructions for the optimal preparation and use of custom Enable-NK™ Activate, after preparation (i.e. 10-fold dilution) described above:

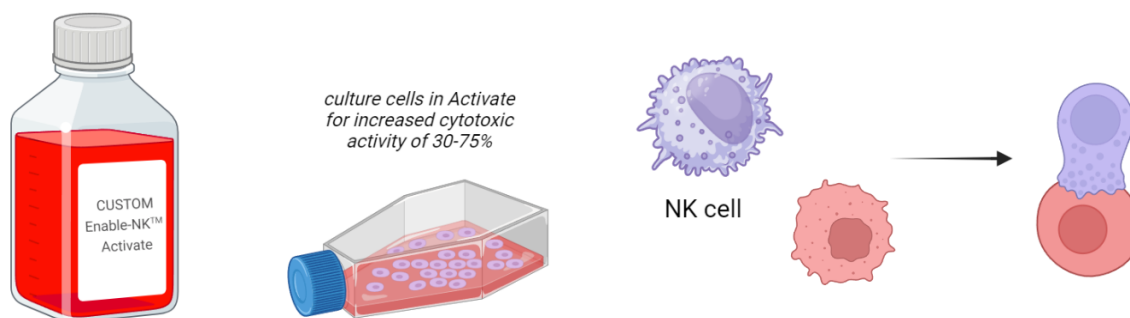


Figure legend: Schematic for the use of Enable-NK™ Activate to maximize NK cell potency against diseased cells.

Additives (additives are not included with product):

- For NK cell lines and primary NK cells:
 - Serum content matching that was used by the end of expansion in Enable-NK™ Grow.
 - IL-2 matching that was used in Enable-NK™ Grow.
 - 1% penicillin/streptomycin
 - Additional additives for **Enable-NK™ Activate 10X Protein-free product version ONLY**:
 - Human serum albumin (HSA), Sigma catalog # A9731
 - Reconstitute and add to achieve a final concentration of 100 µg/mL.
 - Insulin/transferrin/selenite (ITS), Corning catalog # 25-800-CR
 - Add to achieve a 50-fold dilution of ITS into the medium.
 - 4-1BBL, ProSpec catalog # CYT-149
 - Reconstitute and add to achieve a final concentration of 10 ng/mL.
 - Insulin-like growth factor (IGF), ProSpec catalog # CYT-216
 - Reconstitute and add to achieve a final concentration of 20 ng/mL.

Suggestions for culture maintenance:

Cell density

- Suspension cultures (e.g. flasks) should be maintained between $0.75\text{--}2.5 \times 10^6$ cells/mL.
- Cultures using G-Rex plates:
 - Set up at no less than 0.5×10^6 cells/cm².
 - Do not allow culture to exceed 10×10^6 cells/cm².

Duration of culture

- NK cells should be cultured in Enable-NK™ Activate for 2–5 days.

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Caution: Do not “Activate” for too long!

It is recommended to culture the NK cells between 2–5 days in Enable-NK™ Activate. Following these suggestions ensure the product works optimally; however, feel free to experiment with the procedures as desired.

Please note:

- An NK cell expansion protocol WITH feeders has also been developed. Please see **Protocol 2: NK cell expansion using K562 feeder cells and IL-2** on the Suggested Protocols document.
- An IL-2-free NK cell expansion protocol has also been developed. Please see **Protocol 3: NK cell expansion with neither IL-2 nor feeder cells** on the Suggested Protocols document.

Graphics in this document was created using BioRender.com

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