

Enable-NK™ Duo: Instructions for Use

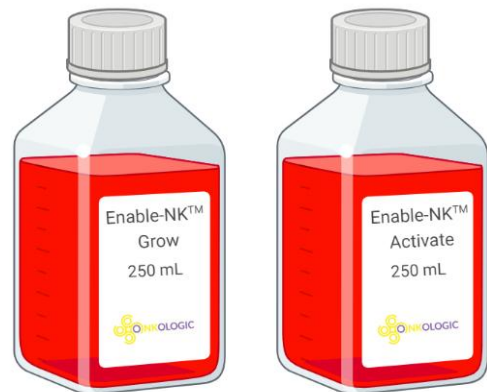
- ❖ **This document provides simple guidelines for optimal use of the product; please refer to the “Suggested Protocols” document for more detailed cell culture methodology information.**

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 is a set of 2 cell culture media consisting of Enable-NK™ Grow and Enable-NK™ Activate, formulated specifically for human natural killer (NK) cells, and is xeno-free. These media are recommended for primary NK cells as well as NK cell lines.

- Enable-NK™ Grow is a cell culture medium for the expansion (proliferation) of NK cells; 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 DMEM/F12 medium and a unique combination of amino acids, fatty acids, vitamins, and other ingredients.
- Enable-NK™ Activate is a cell culture medium 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, deliberately comprising two distinct formulations, is specifically designed for separating the expansion phase from the activation phase, thus avoiding development of an exhaustion phenotype.

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

Enable-NK™ Duo: Instructions for Use

Instructions for the optimal preparation and use of Enable-NK™ Grow:

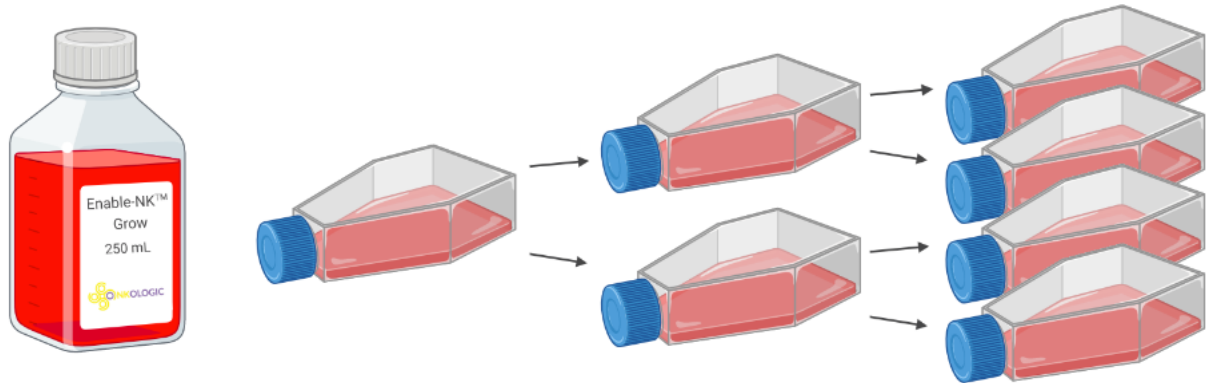


Figure legend: Enable-NK™ Grow has been optimized for expansion of NK cells, for e.g. by splitting using T-flasks.

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 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 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: Instructions for Use

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

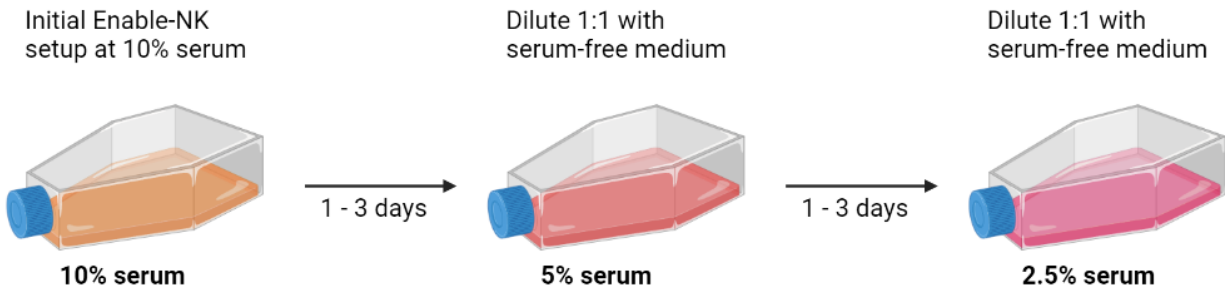


Figure legend: Schematic for suggested stepwise reduction of serum content over 3 to 7 days.

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 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 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: 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.

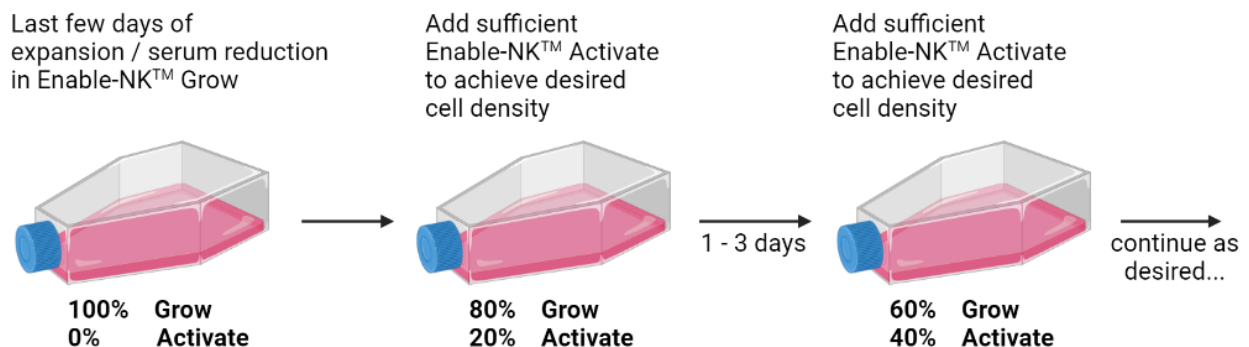


Figure legend: Schematic for gradual transition from Grow to Activate without centrifugation of cells.

Enable-NK™ Duo: Instructions for Use

Instructions for the optimal preparation and use of Enable-NK™ Activate:

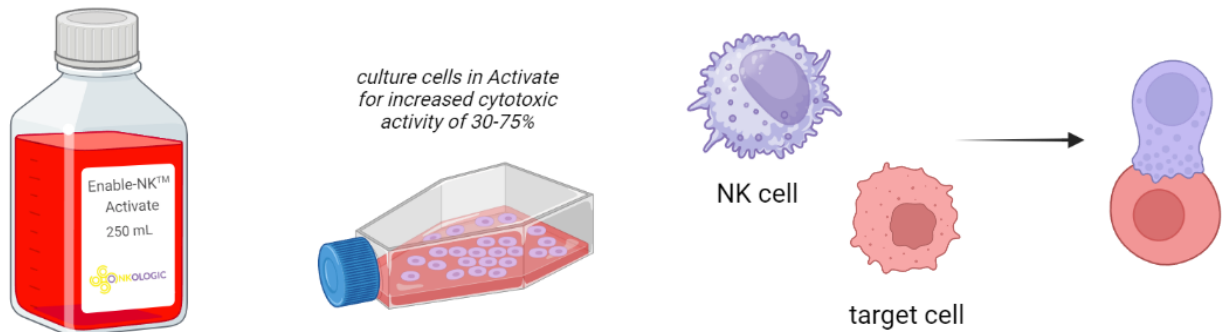


Figure legend: Schematic for the use of Enable-NK™ Activate to maximize NK cell potency against diseased cells.

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 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.

Enable-NK™ Duo: Instructions for Use

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 were created using BioRender.com

Contact Information of Legal Manufacturer

Onkologic INC.
17 Briden Street, Worcester, MA 01605
508-216-0009
contact@onkologic.com