CTS™ NK-Xpander™ Medium

Catalog Numbers A5019001, A5019002

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Gibco™ CTS™ NK-Xpander™ Medium is specifically formulated for the growth and expansion of enriched human Natural Killer (NK) cells in a feeder-free system. It is manufactured without cytokines and growth factors to allow the researcher flexibility for the intended application.

Contents and storage

Contents	Amount	Storage	Shelf Life[1]
CTS™ NK-Xpander™ Medium[2] (Cat. No. A5019001)			
CTS™ NK-Xpander™ Basal Medium	500 mL	2–8°C. Protect from light.	12 months
CTS™ NK-Xpander™ Supplement (50X)	10 mL	-20°C to -5°C	
CTS™ NK-Xpander™ Medium[2] (Cat. No. A5019002)			
CTS™ NK-Xpander™ Basal Medium	5 L (Media Bag)	2–8°C. Protect from light.	12 months
CTS™ NK-Xpander™ Supplement (50X)	100 mL	-20°C to -5°C	

[1] Shelf-life duration is determined from Date of Manufacture.
[2] CTS™ NK-Xpander™ Medium is sold as a complete kit. Individual components are not sold separately.

Culture conditions

Media: Complete CTS™ NK-Xpander™ Medium

Culture type: Stationary suspension

Culture vessels: Non-tissue culture treated 96-well, 48-well, 24-well, 12-well, 6-well plates, T-flasks or cell culture bag.

Temperature range: 36°C to 38°C

Incubator atmosphere: 95% humidified atmosphere of 4–6% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Procedural guidelines

- Thaw CTS™ NK-Xpander™ Supplement (50X) at ambient temperature for 1–2 hours before use. Some small solute may be visible after thaw. Once supplement reaches ambient temperature, mix gently by inverting the bottle 8–10 times. After mixing, no solute should be visible.
- Use thawed material immediately or aliquot (i.e., 1 mL) unused material and store at -20°C to -5°C. Avoid additional freeze-thaw cycles.
- Complete CTS™ NK-Xpander™ Medium (CTS™ NK-Xpander™ Basal Medium, CTS™ NK-Xpander™ Supplement (50X) and 5% Human AB Serum) is stable for 3 weeks when stored in the dark at 2°C to 8°C.
- During NK cell expansion, do not disturb the cells for the first 5 days. After 5 days, do not remove the medium. Add fresh complete CTS™ NK-Xpander™ Medium with fresh Human IL-2 Recombinant Protein (500U/mL).
- After day 5, fresh complete CTS™ NK-Xpander™ Medium with fresh Human IL-2 Recombinant Protein (500U/mL) may need to be added every 1–2 days.
- NK cells may attach to plates or flasks. A cell scraper may be used for non-tissue culture treated T-flasks. Pipette up and down gently after scraping any adherent cells off the flask surface. Vigorous pipetting and foaming can induce unwanted cell death.

Prepare complete CTS™ NK-Xpander™ Medium (500 mL)

1. Aseptically add 10 mL of thawed CTS™ NK-Xpander™ Supplement (50X) to 465 mL of CTS™ NK-Xpander™ Basal Medium.

2. Aseptically add 25 mL of Human AB Serum to medium from step 1 (final concentration of Human AB Serum is 5%). Thoroughly mix by inverting the bottle several times.

3. Immediately before use in culture, supplement complete CTS™ NK-Xpander™ Medium with cytokines or growth factors for extended support of NK cells.

   Note: For NK cell culture, we recommend adding Human IL-2 Recombinant Protein (500U/mL).

4. Complete CTS™ NK-Xpander™ Medium without cytokines can be stored for 3 weeks in the dark at 2–8°C. Make sure cytokines are added immediately before use in culture.

   Note: Ratios provided are for 500 mL of complete CTS™ NK-Xpander™ Medium. Volumes may be scaled up to 5 L as needed by the user.

Use complete CTS™ NK-Xpander™ Medium

This guidance is for static NK cell cultures. For high-density culture in bioreactors, optimal procedures should be determined empirically by the investigator.

1. Equilibrate complete CTS™ NK-Xpander™ Medium to ambient temperature.

2. Immediately prior to use in culture add cytokines. We recommend Human IL-2 Recombinant Protein (500U/mL).

3. Thaw a vial of cryopreserved human peripheral blood mononuclear cells (PBMCs) in a 37°C water bath until a small amount of ice remains.

4. Using a pipette, transfer the entire contents of the cryovial into an empty conical tube.

5. Carefully add 5–10 mL of room temperature CTS™ DPBS without calcium chloride, without magnesium chloride to the conical tube dropwise.

6. Centrifuge the cells at 300 × *g* for 10 minutes at room temperature.

7. Enrich for NK cells using a commercially available NK cell enrichment kit.

8. Suspend enriched NK cells in a minimal volume of complete CTS™ NK-Xpander™ Medium with Human IL-2 Recombinant Protein added.

9. Determine the cell density using the preferred cell counting method.

10. Add complete CTS™ NK-Xpander™ Medium to enriched NK cells for a final cell density of 1.25×10^5 cells/mL.

    Note: Plating cell density may need to be optimized when starting in larger vessels, such as 6-well plates or T-flasks.

11. Add enriched NK cells to a suitable cell culture vessel, for example, to a well of a non-tissue culture treated plate. We recommend starting in a non-tissue culture treated, 96-well, round bottom plate, 200 µL per well.

12. Incubate at 37°C and 5% CO_2.

13. At day 5, pipette cell suspension up and down gently to break up clumps, if any.

14. Determine cell density using the preferred cell counting method.

15. Add complete CTS™ NK-Xpander™ Medium so the final density of cells is between 4.0×10^5– 5.0×10^5 cells/mL. Transfer to a larger cell culture vessel as needed, for example, from a 96-well plate to a 48-well plate or from a 48-well plate to a 24-well plate.

    Note: If the cell density has not reached 4.0×10^5– 5.0×10^5 add fresh medium to replenish cytokines. Transfer to a larger cell culture vessel if needed.

16. For NK cell expansion, addition of complete CTS™ NK-Xpander™ Medium with fresh cytokines is required regularly. After day 5, complete CTS™ NK-Xpander™ Medium with fresh cytokines may need to be added every 1–2 days.

    Note: NK cell expansion varies by donor. Best results are achieved if NK cells are maintained at a density of 0.5–1.0×10^6 cells/mL. Continue to monitor cells daily and add complete CTS™ NK-Xpander™ Medium with fresh cytokines as needed for up to 21 days.

17. Perform flow cytometry analysis to confirm cell phenotype.

Table 1 Suggested flow cytometry antibodies

Product[1]
CD56 Monoclonal Antibody (CMSSB)
CD3 Monoclonal Antibody (OKT3)
CD16 Monoclonal Antibody (CB16)
Live/Dead™ Fixable Dead Cell Stain Kit

[1] Available with multiple fluorophores

- To find antibodies, reagents, protocols, and support for flow cytometry, see **https://www.thermofisher.com/us/en/home/lifescience/cellanalysis/flow-cytometry.html.**
- To build a custom antibody panel using the Flow Cytometry Panel Builder, see **https://www.thermofisher.com/order/panel-builder**

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates	268200
Nunc™ Non-Treated Multidishes, 6-well plates	150239
Nunc™ Non-Treated Multidishes, 12-well plates	150200
Nunc™ Non-Treated Multidishes, 24-well plates	144530
Nunc™ Non-Treated Multidishes, 48-well plates	150787
Nunc™ Non-treated T-25EasYFlask™, Filter Cap	169900
Nunc™ Non-treated T-75 EasYFlask™, Filter Cap	156800
Nunc™ Non-treated T-175 EasYFlask™, Filter Cap	159926
CTS™ DPBS without calcium chloride, without magnesium chloride	A1285601
Human IL-2 Recombinant Protein	PHC0023
Human AB Serum	BP2525100
Countess™ 3 Automated Cell Counter	AMQAX2000
Trypan Blue Solution, 0.4%	15250061
Attune™ NxT Acoustic Focusing Cytometer	A24858[1]

[1] Additional laser configurations are available.

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