

hTC Culture Medium

for primary human T cells

Product information

Content	VZB-1002	500 ml hTC Culture Medium
	VZB-4002	4x500 ml hTC Culture Medium
Shipping and storage	hTC Culture Medium is shipped at room temperature and should be stored at 4°C.	

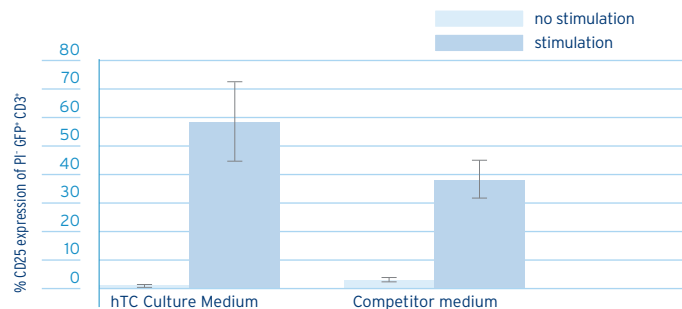
General product use

The hTC Culture Medium provides the optimal nutritional environment for T lymphocyte culture. It has been evaluated to provide high cell viability and transfection efficiency for transfected T cells. The hTC Culture Medium is especially suited for activation experiments post transfection.

hTC Culture Medium provides ideal conditions for activation experiments.

Fig. 1: 20% increased activation with hTC Culture Medium.

Human T cells transfected with 2 µg pmaxGFP™ were activated with anti-CD3/anti-CD28 antibodies 5h post nucleofection. Nucleofected T cells were either cultured in hTC Culture Medium or in a competitor medium optimized for lymphocyte culture. 48h post nucleofection activation was determined by expression of IL2R α chain (CD25).



1

General remark

For nucleofection® of unstimulated human T cells, please refer to the corresponding Optimized Protocol.

2

Preparation of culture medium

Please add 2 mM glutamine (5 ml of a 100x stock) and 10% FCS (50 ml) to 500 ml hTC Culture Medium. Medium supplemented with glutamine and FCS can be stored at 4°C for up to 2 weeks (alternatively it can be frozen in aliquots).

3

Isolation of primary human T cells

Blood samples

Fresh human blood treated with an anticoagulant (e.g. heparin, citrate, ACD-A) or alternatively, leukocyte-enriched buffy coat not older than 8 hours. The samples should be diluted with 2-4 volumes of PBS containing 0.5% BSA (PBS/BSA).

Preparation of PBMC

1. Pipet 15 ml Ficoll-Paque™ Plus [Amersham Pharmacia Biotech AB; Cat. No. 17-1440-03] in a 50 ml conical tube. Overlay Ficoll-Paque™ Plus with 35 ml blood sample and centrifuge at 750xg for 20 minutes at 20°C in a swinging-bucket rotor without brake.
2. Remove the upper layer leaving the mononuclear cell layer undisturbed at the interphase. Carefully transfer the interphase cells (lymphocytes and monocytes) to a new 50 ml conical tube.
3. Add PBS/BSA to 50 ml mark, mix and centrifuge at 350xg for 10 minutes at 4°C. Remove the supernatant carefully.
4. Resuspend the cell pellet in 25 ml of PBS/BSA and centrifuge at 160xg for 15 minutes at 4°C. Remove the supernatant carefully.
5. Resuspend the cell pellet in 25 ml PBS/BSA and centrifuge at 300xg for 10 min at 4°C. Remove the supernatant carefully.
6. Resuspend cell pellet in 5 ml PBS/BSA. Adjust to a concentration of 5×10^7 cells per ml with PBS/BSA.

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Use of hTC Culture Medium for T cell culture

Seed 5×10^6 T cells in 1 ml pre-equilibrated (at least 30 min at 37°C/5% CO₂) hTC Culture Medium supplemented with glutamine and FCS (see above) in a 12-well plate. Unstimulated T cells can be cultured for up to 48h in hTC Culture Medium. After a culture period of 24h - 48h we recommend to feed cells with additional 0.5 ml fresh supplemented hTC culture medium.

For culture longer than 48 h it might be useful to add IL-2 to the supplemented hTC Culture Medium.

5

Use of hTC Culture Medium for culture of nucleofected T cells

Please follow the instructions given in the Optimized Protocol for unstimulated human T cells.

Incubate the nucleofected cells in pre-equilibrated (at least 30 min at 37°C/5% CO₂) hTC Culture Medium in 12-well plates **for 5-6 h**. After this time, spin the plates for 8 min at 140xg in a tissue culture centrifuge, carefully remove the medium and add fresh pre-equilibrated hTC Culture Medium. A medium change at 5-6h after nucleofection leads to an increased viability after transfection. Continue as indicated in the Optimized Protocol.

6

Use of hTC Culture Medium for stimulation of nucleofected T cells

Please follow the instructions given in the Optimized Protocol for unstimulated human T cells. Incubate the nucleofected cells in pre-equilibrated hTC Culture Medium in 12-well plates **for 5-6 h**. After this time, spin the plates for 8 min at 140xg in a tissue culture centrifuge, carefully remove the medium and add fresh pre-incubated hTC Culture Medium containing **anti-CD3/anti-CD28 antibodies**. Alternatively, replace medium and seed 1/5 of the cells in 96-well **MaxiSorb™ plates** coated with these antibodies. Continue as indicated in the Optimized Protocol.

For optimal **stimulation** of the nucleofected cells we recommend coating 96-wells (NUNC Immuno™ Plate C96 MaxiSorb™, Cat.No. 430341) with 50 µl of a solution of anti-CD3 antibody (OKT3, eBioScience, Cat.No. 14-0037-82; final conc. 1 ng/µl) and anti-CD28 antibody (15E8, Research Diagnostics Inc, Cat.No. RD1-M1650cIb; final conc. 2 ng/µl), or with a solution of a control antibody (purified mslgG(κ), BD-Pharmingen Cat.No. 55472; final conc. 3 ng/µl). After incubation for 2 h at 37°C/5% CO₂ cells are washed three times with PBS containing 0.5% (w/v) BSA. Antibodies should be diluted in carbonate buffer (32 mM Na₂CO₃/16 mM NaHCO₃) from 100 ng/µl stock solutions directly before use.

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