GENAXXON bioscience

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Serum Replacement NTA

Defined Serum Substitute for Adherent Cells

Component	Cat#	C4369.0050	C4369.0100	C4369.0500
Serum Replacement NTA a defined serum substitute for adherent cells		50mL	100mL	500mL

Product Description
Serum Replacement NTA is a ready-to-use, fully defined serum substitute for the cultivation of adherent cells under serum-free conditions. This sterile product can be used at the standard concentration of 10% in the cell culture. It supports the adherent growth of many cell types in an optimum manner.

Composition
Serum Replacement NTA contains purified proteins (<2% w/v), lipids, salts, amino acids, trace elements, attachment factors, hormones and traces of animal-derived components (< 2% w/v). Serum Replacement NTA contains no growth factors, undefined hydrolysates or peptones.

Special advantages
Serum Replacement NTA is designed to replace or to reduce serum in the cell culture in a very simple manner. In most cases there is no need to change the basal medium. As Serum Replacement NTA is fully defined and contains no peptones or hydrolysates, lot testing is no more necessary.

- It also allows high reproducibility and a simplified downstream process.
- Serum Replacement NTA contains no growth factors and enables defined proliferation and differentiation of stem cells.
- Characterization studies of growth factors will obtain more reproducible and clearer results.

Serum Replacement NTA is also useful to develop sensitive cell-based in vitro tests and co-culture procedures. For cell lines which require specific growth factors these should be added in a concentration as previously used.

Suitability
Serum Replacement NTA is suitable for the cultivation of a variety of adherent cells under serum-free culture conditions (please see figure 1) or to reduce the necessary FBS amount in cell culture.

Effect of Serum Replacement NTA supplement on different cell lines

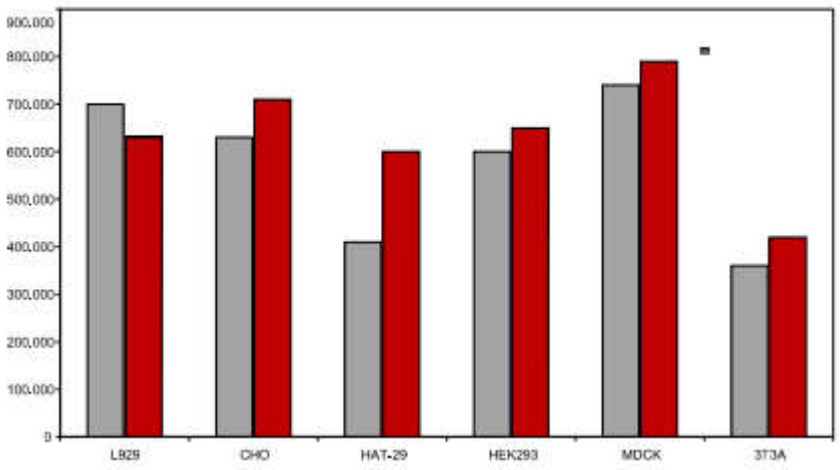

Fig. 1: Efficiency and growth stimulation of Serum Replacement NTA compared to FBS (10% each in DMEM/F12) at 7 day culture in different cell types

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Instructions for use:
Serum Replacement NTA can be stored and used in the same manner as serum.

- **Thaw Serum Replacement NTA** at maximum 37°C. **Please avoid repeated freeze-thaw cycles!**
- **To replace serum:** Use the same basal medium and the same concentration of Serum Replacement NTA as FBS. The performance can be further improved by optimizing the concentration of Serum Replacement NTA or modifying/changing the basal medium (Table 1)
- **To reduce serum concentration:** Use the same basal medium and add the same amount of Serum Replacement NTA as the reduced amount of serum, until the minimal necessary concentration of FBS is found (1 to 2.5% in most cases). The performance can be further improved by optimizing the concentration of Serum Replacement NTA or modifying/changing the basal medium (also see adaptation instruction and table 1).
- **Recommended inoculation** cell density: 5.000 – 20.000 cells/cm^2.
- **Solve cells as usual** from the cell culture vessel (e.g., Trypsin 0.25%/EDTA 0.02% in PBS, or Accutase®). Once the cells have become round and detach from the surface inactivate trypsin with trypsin inhibitor: Simply resuspend cells in about 1mL trypsin inhibitor solution for every ml of trypsin solution used for dissociation**. Note** that Accutase® does not need to be inhibited.

Depending on the cell type, some differences in morphology or proliferation rate may be observed with varying standard media. Most applications were performed with DMEM and DMEM/F12 for adherent cells. Make sure that L-glutamine is present in sufficient quantity. The optimal Serum Replacement NTA concentration should be determined for each cell line. Tests can be started at Serum Replacement NTA concentration of 10%, as with most cells the best results were obtained at this concentration.

Please note: For more demanding cells an adaptation to Serum Replacement NTA may be necessary.

Adaptation instructions for Serum Replacement NTA
Precondition for a successful transition are vital cells (trypan blue exclusion staining), which should be harvested in the logarithmic growth phase. If 10% FBS was used in the original protocol.

Step 1: 7.5% FBS + 2.5% Serum Replacement NTA
- Seed cells at 5 x 10^3 – 20 x 10^3 ells/cm^2.
- Observe cells under a microscope, at about 90 % confluence passage the cells for another 2-3 passages.

If normal growth is obtained transfer cells into:

Step 2: 5% FBS + 5% Serum Replacement NTA
- Seed cells at 5 x 10^3 – 20 x 10^3 cells/cm^2.
- Observe cells under a microscope, at about 90% confluence passage the cells for another 2-3 passages.

If normal growth is obtained transfer cells into:

Step 3: 2.5% FBS + 7.5% Serum Replacement NTA
- Seed cells at 5 x 10^3 – 20 x 10^3 cells/cm^2.
- Observe cells under a microscope, at about 90% confluence passage the cells for another 2-3 passages.

If normal growth is obtained transfer cells into:

Step 4: 1% FBS + 9% Serum Replacement NTA
- Seed cells at 5x10^3 – 20x10^3 cells/cm^2.
- Observe cells under a microscope, at about 90% confluence passage the cells for another 2-3 passages.

If normal growth is obtained transfer cells into:

Step 5: 10% Serum Replacement NTA
- Seed cells at 5 x 10^3 – 20 x 10^3 cells/cm^2.
- Observe cells under a microscope.

For some cells an adaptation to serum-free conditions is difficult to reach or even impossible. The following measures may help to facilitate a successful adaptation:

- Reseeding with a higher cell amount (about 2x to 4x of the usual cell density).
- Addition of growth factors (if known, which factors have a positive effect on the relevant cells).
- Coating the culture dishes or flasks with attachment factors (e.g., fibronectin, laminin, collagen, gelatine, etc.).
- Change the basal medium. **Note:** A change of the basal medium to a richer or more complex formulation may be all that is needed to achieve growth in serum free condition.

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Tabel 1: Comparison of Cell Growth in 10% Serum Replacement NTA in different Basal Media versus Cell Growth in 10% FBS in different Basal Media

Cell Line	Origin	Basal Medium	Percentage Growth 10% Serum Replacement NTA	Percentage Growth 10% FBS
HEK 293 T	Renal cells, human embryonic	DMEM/F12 alpha-MEM DMEM	105% 76% 62%	100%
MDCK	Renal cells, canine	DMEM/F12 alpha-MEM McCoy's 5A	102% 91% 106%	100%
MDBK	Renal cells, bovine	RPMI 1640 McCoy's 5A DMEM	122% 135% 131%	100%
L 929	Fibroblasts, mouse	DMEM RPMI 1640 Ham's F-12	97% 78% 128%	100%
HT-29	Colon Carcinoma, human	IMDM DMEM/F12 alpha-MEM	108% 98% 96%	100%
HeLa S3	Cervix carcinoma epithel, human	Glasgow MEM IMDM EMEM	106% 72% 100%	100%
CHO	Ovarial cells epithel, Chinese hamster	DMEM/F12 alpha-MEM IMDM	106% 97% 82%	100%
CHO-Luc	Ovarial cells epithel, Chinese hamster, transfected	IMDM DMEM alpha-MEM	86% 97% 84%	100%
3T3A	Fibroblasts, mouse	RPMI 1640 McCoy's 5A DMEM/F12	98% 72% 97%	100%
MCF-7	Mammary carcinoma, human	Ham's F-12 DMEM/F12 RPMI 1640	292% 176% 214%	100%
RAW 264.7	Macrophages, mouse	McCoy's 5A DMEM/F12 alpha MEM	40% 67% 38%	100%

Storage conditions
Storage: -20°C (in the dark)
Stability: 2 years from date of production

Genaxxon can also offer Serum Replacement NTA Pharma grade. This is the xeno-free version of Serum Replacement NTA. Customized formulations (e.g., without hormones and insulin) on request.