

Datasheet

Panexin NTA

Defined Serum Substitute for Adherent Cells

| Product | Description | Catalogue-No. | Size |
|-------------|---|---------------|--------|
| Panexin NTA | Defined serum substitute for adherent cells | P04-95070 | 50 ml |
| | | P04-95700 | 100 ml |
| | | P04-95750 | 500 ml |

Product description

Panexin 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.

Storage conditions

Storage: -20°C (in the dark)

Stability: 2 years from date of production

Size: 50 ml, 100 ml, 500 ml, other sizes on request

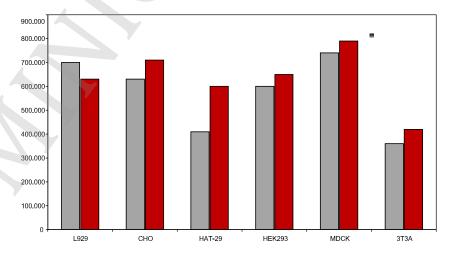
Composition

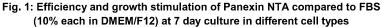
Panexin NTA contains purified proteins (< 2 % w/v), lipids, salts, amino acids, trace elements, attachment factors, hormones and traces of animal-derived components (< 2% w/v). Panexin NTA contains no growth factors, undefined hydrolysates or peptones. Panexin NTA Pharma grade is the xeno-free version of Panexin NTA. Customized formulations (e.g. without hormones and insulin) on request.

Suitability

Panexin 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 Panexin NTA in different cell lines





■ FBS

Panexin NTA



Special advantages

Panexin 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 Panexin 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. Panexin 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. Panexin 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.

Instructions for use

Panexin NTA can be stored and used in the same manner as serum.

- Thaw Panexin at maximum 37 °C. Please avoid repeated freeze-thaw cycles!
- <u>To replace serum</u>: Use the same basal medium and the same concentration of Panexin as FBS. The performance can be further improved by optimizing the concentration of Panexin or modifying/changing the basal medium^a (Table 1)
- <u>To reduce serum concentration</u>: Use the same basal medium and add the same amount of Panexin 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 the concentration of Panexin or modifying/changing the basal medium^a (also see adaptation instruction and table 1).
- Recommended inoculation cell density: 5.000 20.000 cells/cm².
- Solve cells as usual from the cell culture vessel (e.g. Trypsin 0.25 %/EDTA 0.02 % in PBS, Cat.No. P10-020100 or Accutase®, Cat.No. P10-21100). Once the cells have become round and detach from the surface inactivate trypsin with trypsin inhibitor (Cat.No. P10-034100): Simply resuspend cells in about 1 ml 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 Panexin NTA concentration should be determined for each cell line. Tests can be started at a Panexin 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 Panexin NTA may be necessary.



Adaptation instructions for Panexin 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 % Panexin

- Seed cells at 5 x 10³ 20 x 10³ cells/cm².
- 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 % Panexin

- Seed cells at 5 x 10³ 20 x 10³ cells/cm².
- 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 % Panexin

- Seed cells at 5 x 10³ 20 x 10³ cells/cm².
- 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 % Panexin

- Seed cells at 5x10⁴ 10x10⁵ cells/ml.
- 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 % Panexin

Seed cells at 5 x 10³ – 20 x 10³ cells/cm².

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.



Table 1: Comparison of Cell Growth in 10% Panexin NTA in different Basal Media versus Cell Growth in 10% FBS in different Basal Media

| Cell Line | Origin | Basal Medium | Percentage of Growth 10% Panexin NTA | Percentage of Growth 10% FBS | |
|-----------|------------------------|-----------------|--|------------------------------------|--|
| HEK 293 T | Renal cells, human | DMEM/F12 | 105% | 100% | |
| | embryonic | alpha-MEM | 76% | 10076 | |
| | | DMEM | 62% | | |
| MDCK | Renal cells, canine | DMEM/F12 | 102% | 100% | |
| | | McCoy's 5A | 91% | 100% | |
| | | alpha-MEM | 106% | | |
| MDBK | Renal cells, bovine | RPMI 1640 | 122% | 4000/ | |
| | | McCoy's 5A | 135% | 100% | |
| | | DMEM | 131% | | |
| L 929 | Fibroblasts, mouse | DMEM | 97% | 1000/ | |
| | | RPMI 1640 | 78% | 100% | |
| | | Ham's F-12 | 128% | | |
| HT-29 | Colon Carcinoma, | IMDM | 108% | 4000/ | |
| | human | DMEM/F12 | 98% | 100% | |
| | | alpha-MEM | 96% | | |
| HeLa S3 | Cervix carcinoma | Glasgow MEM | 106% | 4000/ | |
| | epithel, human | IMDM | 72% | 100% | |
| | • | EMEM | 100% | | |
| СНО | Ovarial cells epithel, | DMEM/F12 | 106% | 40001 | |
| | Chinese hamster | IMDM | 97% | 100% | |
| | | alpha-MEM | 82% | | |
| CHO-Luc | Ovarial cells epithel, | IMDM | 86% | 10.531 | |
| | Chinese hamster, | DMEM | 97% | 100% | |
| | transfected | alpha-MEM | 84% | | |
| 3T3A | Fibroblasts, mouse | RPMI 1640 | 98% | 4000/ | |
| | | McCoy's 5A | 72% | 100% | |
| | | DMEM/F12 | 97% | | |
| MCF-7 | Mammary | Ham's F-12 | 292% | 4000/ | |
| | carcinoma, human | DMEM/F12 | 176% | 100% | |
| | | RPMI 1640 | 214% | | |
| RAW 264.7 | Macrophages, | McCoy's 5A | 40% | 100% | |
| | mouse | DMEM/F12 | 67% | | |
| | | alpha-MEM | 38% | | |

Technical support

For technical support, questions or remarks please contact your local PAN-Biotech partner or the technical department of PAN-Biotech via email (info@pan-biotech.com) or phone +49-8543-601630.

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^a As a basal medium, standard media such as RPMI 1640, DMEM (high or low glucose), DMEM/F12, IMDM etc. can be used. Make sure that L-glutamine is present in sufficient quantity (supplement L-glutamine as needed).