



## Frequently Asked Questions

### What is BioNuesis?

BioNuesis is naturally soluble human extracellular matrix (hECM) from hypoxic cultures of human dermal fibroblast feeder layer cells, grown in tissue-engineered 3-dimensional bioreactors. Our scientists discovered that the hECM proteins, laminins, collagen type IV, and fibronectin are secreted into our proprietary, defined serum free medium. Western blotting and functional assays have demonstrated that these hECM proteins in the cell-conditioned medium are intact, and not degraded, and retain bioactivity. Because BioNuesis contains naturally soluble hECM proteins, it does not polymerize.

### What does BioNuesis contain?

It contains natural soluble hECM proteins including: laminin, collagen type IV, and fibronectin. We have also detected a number of other hECM proteins, including tenascin C, and other collagen types, but these are at lower apparent levels. We are actively characterizing all the cell-secreted factors found in BioNuesis in collaboration with academic partners, with the goal of defining the entire composition to the standards required by the FDA for biologics products.

### What is the intended use of BioNuesis kits?

BioNuesis soluble hECM is intended for the adherence and expansion of cells in culture, including stem and progenitor cells, such as human ES cells. Many other cell types which require the ECM proteins laminin, fibronectin, or collagen type IV can also be cultured with BioNuesis. BioNuesis is intended for research purposes only.

### How much do you get out of one BioNuesis kit?

A BioNuesis kit comes as 2 mls of liquid that is to be diluted 20 fold in buffered salt solution. This provides 40 mls of “coating solution” per kit. Use 1 ml per well of a 6 well plate, or use 4 mls per 10 cm petri dish.

### Is BioNuesis stable?

BioNuesis is very stable and can be stored at 4-10°C for up to three months. For longer-term storage it is recommended that BioNuesis be frozen at -20°C degrees for a period of up to 6 months when not diluted. Avoid repeated freeze-thawing, but a single-freeze thaw step does not reduce the activity in cell culture.

### What cell types has this been tested on?

We have tested BioNuesis kits on the well-characterized human embryonic stem cell line BG01v, in comparison to Matrigel-coated cultures, in StemPro and mTeSR media supplemented with 40 ng/ml bFGF. Independent academic institutions are currently evaluating BioNuesis on other huES cell lines and other stem cell types.

### What quality control standards are currently in place?

Currently BioNuesis kits are being produced under a pilot cGMP process to ensure product consistency while we progress towards GMP certification of our manufacturing plant. Our current lot size produces 2500 kits in each lot. BioNuesis kits are assayed for human laminin subunit gamma-1 by ELISA, and are aseptically-filled after sterile filtration and sterility checked. The human fibroblast cell line has been mycoplasma tested, and has also tested negative for HIV, Hepatitis B, and Hepatitis C.

