

# NutriStem® V9 XF

A defined, xeno-free, serum-free culture medium for hPSC using vitronectin



NutriStem® V9 XF is a defined, xeno-free, serum-free medium designed to support the growth and expansion of human pluripotent stem cells (hPSC), using vitronectin and enzyme-free passaging as small aggregates. NutriStem® V9 XF medium contains only the essential components required for long-term maintenance of hPSC. This medium shows superior proliferation rates during long-term cultures, while maintaining the pluripotency of the cells.

NutriStem® V9 XF medium allows the culture of hPSC in vitronectin pre-coated culture, as well as the direct addition of vitronectin to the medium (no need for precoating).

- Superior results using vitronectin
- Precoating-free option
- Defined, serum-free, xeno-free
- Produced under cGMP
- Supports long-term expansion
- High pluripotency at high passages
- Weekend-free
- Biological relevant GF concentration
- Cytokine-free basal medium, applicable for EBs formation, reprogramming, and differentiation

### Figure 1: Cell count during long-term expansion

Nucleocounts performed on equal volume of cell suspension (Chemometech, count of aggregated cells) during long-term expansion of H1 hESC cultured in NutriStem® V9 XF medium and E8 under a weekend-free feeding regime using 0.5µg/cm<sup>2</sup> Vitronectin ACF.



NutriStem® V9 XF medium shows superior proliferation rates in long-term culture (20 passages)

# Figure 2: Typical hPSC colony morphology during long-term culture

Phase contrast images (x100) of H1 hESC culture maintained in NutriStem® V9 XF medium under a weekend-free feeding regime, using 0.5µg/cm<sup>2</sup> Vitronectin ACF. Representative images from culture at P6, P16 and P20.



Undifferentiated colonies maintained during long-term culture of H1 hESC

# Figure 3: Flow cytometric quantification

Immunophenotyping analysis for human pluripotent markers of H1 hESC culture maintained in NutriStem® V9 XF medium under a weekend-free feeding regime using 0.5µg/cm<sup>2</sup> Vitronectin ACF at P8 and P17. Data presented as % expression from gated viable cells.



High expression of pluripotent stem cell markers in cells cultured in NutriStem® V9 XF medium on vitronectin

## Figure 4: Immunofluorescence staining

Immunofluorescence analysis of human pluripotent markers of H1 hPSC expanded in NutriStem® V9 XF medium under a weekend-free feeding regime using Vitronectin ACF. Cells from P8 were fixated and stained for the classic pluripotent surface markers: TRA 1-60 (Alexa Fluor) (green) and nuclear conjugated markers: Nanog-RRX, counterstained with DAPI (blue). Scale bar 200µm.



Cells cultured in NutriStem® V9 XF Medium on vitronectin express high levels of pluripotent markers

## Figure 5: Confirmation of tri-lineage differentiation potential by gene expression

Embryoid bodies (EBs) were generated from H1 hES cells from P18 in NutriStem® V9 XF medium under a weekendfree feeding regime on 0.5µg/cm<sup>2</sup> Vitronectin ACF as an evaluation of pluripotency. Cells were suspended in NutriStem® V9 XF basal medium, where they spontaneously formed EBs containing cells of embryonic germ layers. Real-time PCR analysis for human pluripotent and differentiation genes from the 3 germ layers calibrated to ACTB2 and GAPDH. Results presented as % expression from H1 hES cells before long-term expansion.



NutriStem® V9 XF medium maintained hESC tri-lineage germ layer differentiation potential

# **Precoating-Free Procedure**

The use of a recombinant protein matrix, such as vitronectin, markedly improves stable maintenance of hPSCs in serum-free conditions. However, its use is time-consuming and laborious. Therefore, a friendly-to-use protocol has been developed to eliminate the precoating procedure. While seeding, Vitronectin ACF can be added directly into NutriStem® V9 XF medium, making pre coating unnecessary.

**Figure 6:** H1 hESC cultured in NutriStem® V9 XF medium for 6 sequential passages under a weekend-free feeding regime using a precoating-free protocol. Vitronectin ACF was added directly to NutriStem® V9 XF medium before cell seeding. Representative colony morphology images of H1 hESC (A), iPSC ACS 1019 (ATCC) (B) (x100), flow cytometric quantification (C).

#### A- H1 Morphology



#### B- iPSC Morphology





NurtiStem<sup>®</sup> V9 XF

The precoating-free protocol supports classic colony morphology while maintaining high levels of pluripotent markers expression

# Figure 7: Immunofluorescence staining

Immunofluorescence analysis of human pluripotent markers of H1 hESC expanded in NutriStem® V9 XF medium under a weekend-free feeding regime using a precoating-free protocol. Cells from P8 were fixated and stained for the classic pluripotent surface markers: SSEA4 (RRX) (red), TRA 1-60 (Alexa Fluor) (green) and nuclear conjugated markers: OCT-4-Alexa Fluor, counterstained with DAPI (blue). Scale bar 200µm.



Cells cultured in NutriStem® V9 XF medium using the precoating-free protocol express high levels of pluripotent markers

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# **Ordering Information**

Cat.#	Product	Size	NutriStem® V9 XF
05-105-1A	NutriStem® V9 XF basal medium	500ml	
05-106-1F	NutriStem® V9 XF supplement mix	1ml	
05-754-0002	Vitronectin ACF	200µg	Contact Us
01-862-1B	0.5M EDTA Solution	100ml	

### How to Order

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