

## Authentic Recombinant Noggin<sup>HuXp</sup> Consistently Suppresses Differentiation of Human Embryonic Stem Cells



### INTRODUCTION

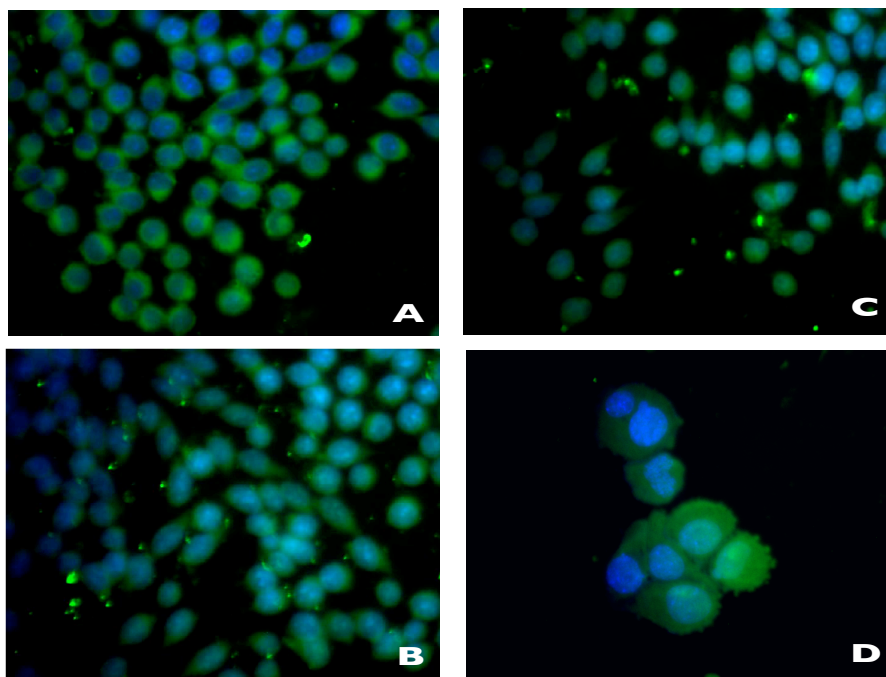
Recombinant human cytokines are essential for the expansion and differentiation of human stem cells. As stem cell research continues to advance and findings are translated to the clinic, there is a growing need for recombinant cytokines to support the clinical development of cellular therapies. HumanZyme, Inc. has developed an efficient human-cell based technology, **HumaXpress™**, for the scalable production of human cytokines. An expanding range of authentic, tag-free cytokines, is available, including difficult-to-express protein members of the TGFβ1 superfamily. HumanZyme's authentic cytokines can be used as cost effective, highly preferred reagents for stem cell research and clinical development.

### Biologically Relevant Noggin

Noggin is a secreted homodimeric glycoprotein that is an antagonist of bone morphogenetic proteins (BMPs). During culture of human embryonic stem (hES) cells without feeder layers

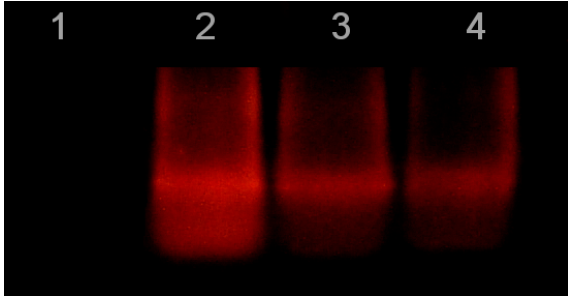
or conditioned medium (but with addition of FGF basic), the addition of Noggin allows the stem cells to maintain their undifferentiated, pluripotent state. Commercially available Noggin products are produced in a variety of forms none of which are authentic: non-glycosylated protein expressed in E coli; glycosylated Fc-fusion protein expressed in NS0, for example. HumanZyme has produced Noggin<sup>HuXp</sup> in a stable, engineered human 293 cell expression system. The protein is expressed as an authentic glycosylated, disulfide-linked dimer.

The bioactivity of Noggin<sup>HuXp</sup> was initially determined by the dose-dependent inhibition of BMP4 induced alkaline phosphate production by ATDC5 cells. More importantly, as shown in the Figure, hES cells in the presence of Noggin<sup>HuXp</sup> exhibit high expression of surface markers typical of undifferentiated stem cells: TRAI-60, TRAI-81, SSEA-4 and transcriptional factors OCT3/4. However, these markers are normally



Expression of pluripotent stem cell markers of hES colonies cultured in the presence of recombinant Noggin<sup>HuXp</sup> expressed in human cells. Noggin concentration 10-20 pg/ml  
 A. Green TRAI-60  
 B. Green SSEA-4  
 C. Green TRAI-81  
 D. OCT3/4  
 Blue = Nuclear in all cases

only inconsistently detectable when recombinant Noggin from non-human cells expressions systems is used at substantially higher concentrations making large scale cell culture un-economical.



#### Western Blot Analysis of OCT3/4

1. Negative Control
2. Positive Control - OCT3/4
3. Human Embryonic Stem Cells Treated with 10 pico grams per ml Noggin<sup>HuXp</sup>
4. Human Embryonic Stem Cells Treated with 20 pico grams per ml Noggin<sup>HuXp</sup>

This product adds to the rapidly expanding range of stem cell related cytokines available from HumanZyme Inc., manufactured to high quality standards and providing high biological activity, lot-to-lot consistency and low endotoxin levels. See product numbers (HZ-1026, HZ-1037, HZ-1085, HZ-1117) Noggin<sup>HuXp</sup> is available in trial size and in bulk.