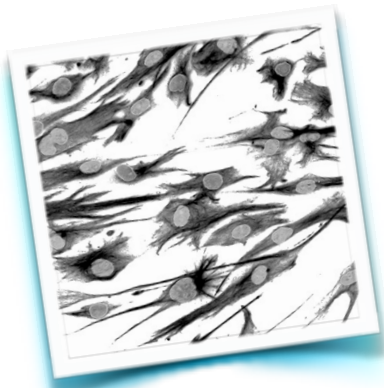


Mesoderm from hPSCs

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Mesoderm Production Protocol

1. Add mesoderm media to hESCs on day 1.
Note: cell density does not seem to be important
2. Assay cells for Brachyury expression on day 3.

Mesoderm media

RPMI

0.5% HyClone FBS BMP4 (10 ng/ml) Wnt3A (50 ng/ml) [we'd likely try CHIR99021 next time]

We have used the Brachyury antibody from R&D Systems (AF2085; 1:40) with success.

Protocol developed by Kathy Joubin in parts from:

Watanabe et al., 2007 *Nature Biotechnology* 25:681-686.

Nakanishi et al., 2009 *FASEB Journal* 23:144-122.

Zhang et al., 2009 *Blood* 111:1933-1941.