

# **Pluripotent Stem Cell Culture Essentials:** *Tips and Tricks*



## Stem cells not forming colonies



Changes in cell morphology



**Uneven or low** attachment to plate





Slow cell division





- Cells handled harshly breaking apart colonies
- Cells have started prematurely differentiating
- Cells are too sparse
- Cells have overgrown
- Cells are responding to stress or physical strain
- Expired media components causing premature differentiation
- Incorrect handling of cells during passaging
- Stem cells have autodifferentiated into other cell types
- Extracellular matrix forming unevenly
- Incorrect extracellular matrix
- Incorrect amount of extracellular matrix
- Y-27632 slowing cell growth
- Media is spent
- Media is missing vital components
- Protocol is not reproducible
- Loss of pluripotency due to stress, or incorrect handling

- Use wide orifice tips and avoid over-pipetting. Centrifuge at low RPM
- Check media / check for contamination, including mycoplasma
- Check pluripotency markers
- Make sure to thaw stem cells at 50-70% confluency based on plate size/recommendations
- Split cells while still in well-defined colonies at ~ 70% confluency
- Avoid over-pipetting
- Change media as per manufacturer's recommendation and check expiration date
- Avoid passaging cells to single cells to avoid cell stress. Use a gentler dissociation enzyme (i.e. Accutase)
- Keep extracellular matrix cold to avoid uneven formation
- Check the best ECM for your purposes
- Vary ECM amount depending on cell type and 2D vs 3D culture
- Make sure you added the right amount of ROCK inhibitor
- Change media as per manufacturer's recommendation
- Verify the media has all the necessary additives like growth factors etc.
- Signs of mycoplasma/other types of contamination. Check media and perform mycoplasma test
- Test other protocols to achieve your intended differentiation
- Check for pluripotency markers before proceeding for differentiation

## differentiating

in previous passages

- Incorrect cytokine/growth factor amount and source
- Check your cytokine specific activity to match amount to use more accurately
- Source cytokines/growth factors expressed in cells that can ensure right post translational modifications

# PROTEINTECH'S PLURIPOTENT STEM CELL CULTURE SOLUTIONS

#### Antibodies to characterize Pluripotent stem cell and germ layer differentiations

ency marker antibod	lies	En	doderm marker antibodi	es
Proteintech Antibodies	Number of citations	Marker	Proteintech Antibodies	Number of citations
14295-1-AP	184	SOX17	24903-1-AP	7
11263-1-AP	197	FOXA2	22474 1 40	1 /
11064-1-AP	212	FOXAZ	22474-1-AP	16
11880-1-AP	106	CXCR4	60042-1-lg	36
	ency marker antibod Proteintech Antibodies 14295-1-AP 11263-1-AP 11064-1-AP 11880-1-AP	ency marker antibodiesProteintech AntibodiesNumber of citations14295-1-AP18411263-1-AP19711064-1-AP21211880-1-AP106	Proteintech AntibodiesNumber of citationsMarker14295-1-AP184SOX1711263-1-AP197FOXA211064-1-AP212CXCR4	Proteintech AntibodiesNumber of citationsMarkerProteintech Antibodies14295-1-AP184SOX1724903-1-AP11263-1-AP197FOXA222474-1-AP11064-1-AP212CXCR460042-1-Ig

Ectoderm marker antibodies		Mesode	rm marker antibodi	es	
Marker	Proteintech Antibodies	Number of citations	Marker	Proteintech Antibodies	Numb citati
Nestin	19483-1-AP	70	NCAM1	14255-1-AP	41
 PAX6	12323-1-AP	49	NKX 2.5	13921-1-AP	12
 OTX2	13497-1-AP	22	 TBX6	12447-1-AP	

Discover more



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### Growth factors for Pluripotent stem cell and its differentiations

Pluripotent stem cell (PSC) maintenance			
Growth factor	Humankine	Number of citations	
bFGF	HZ-1285	50	
TGFb1	HZ-1011	156	

Growth factors for germ layer differentiations			
Growth factor	Proteintech products	Number of citations	
Activin A	HZ-1138	25	
Noggin	HZ-1118	13	
BMP2	HZ-1128	32	
BMP4	HZ-1045	69	
bFGF	HZ-1285	50	

Growth factors for Neural differentiation			
Growth factor	Proteintech products	Number of citations	
bFGF	HZ-1285	50	
EGF	HZ-1326	10	
beta NGF	HZ-1222		
BDNF	HZ-1335		
GDNF	HZ-1311	1	
FGF8B	HZ-1103	3	
CNTF	HZ-1331		
DKK1	HZ-1314		

Growth factors for Cardiac differentiation			
Growth factor	Proteintech products	Number of citations	
Activin A	HZ-1138	25	
BMP4	HZ-1045	69	
bFGF	HZ-1285	50	
VEGF165	HZ-1038	29	
VEGF121	HZ-1204	18	

Growth factors for Pancreatic differentiation			
Growth factor	Proteintech products	Number of citations	
HGF	HZ-1084	14	
BMP4	HZ-1045	69	
IGF1	HZ-1322		
Activin A	HZ-1138	25	

Growth factors for Hepatocyte differentiation			
Growth factor	Proteintech products	Number of citations	
bFGF	HZ-1285	50	
Wnt3A	HZ-1296	2	
HGF	HZ-1084	14	
OSM	HZ-1030	5	
FGF19	HZ-1330		
Rspondin 1	HZ-1328	1	
BMP4	HZ-1045	69	
EGF	HZ-1326	10	