

Materials List for:

Derivation of Stem Cell Lines from Mouse Preimplantation Embryos

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Materials

Name	Company	Catalog Number	Comments
1 mL sryringe	BD	309628	
2-β-mercaptoethanol	Life Technologies	31350-010	
4-well plate	Thermo Scientific Nunc	176740	
Acidic Tyrode's solution	Home-made		See recipe in Nagy et al, 2003. For short-term storage (up to 1 month) keep it at 4°C. Alternatively, for long-term storage keep it at -20°C. If the efficiency of the solution diminishes, it should be acidified by the addition of a small drop of HCl 1M.
BSA	Sigma	A3311	
Chicken anti-Mouse IgG, Alexa Fluor 488	Molecular Probes - Invitrogen	A-21200	1:500 dilution. Secondary antibody for Oct4, Tuj1, αSMA and AFP mouse antibodies.
CHIR990212	Axon Medchem	1386	Reconstitute with 2.15 ml DMSO for each mg of powder, to obtain a 1 mM stock solution. Aliquot at desired volume and store at -20°C. Avoid freeze and thaw cycles.
CryoTube	Thermo Scientific Nunc	3754518	
DMSO	Sigma	41640	
DMEM	BioWest	L0107	
FBS	BioWest	S1810	Inactivate it prior to use by heating for 30 mins at 56°C. Aliquot and store at -20°C
Flushing needle	BD	304000	Cut the end of the needle and ground to a blunt tip on an abrasive stone
Gelatin from porcine skin	Fluka	48724	Dilute at 0,2% in distilled water and autoclave it. Each dilution can be used for a month.
Goat anti-Rabbit IgG, Alexa Fluor 594	Molecular Probes - Invitrogen	A-11037	1:500 dilution. Secondary antibody for Sox2 rabbit antibody.
HBSS	BioWest	L0611	
Hepes-buffered CZB	Home-made		See composition in Chatot et al, 1989
Human chorionic gonadotropin	Divasa-Farmavic	Veterin-Corion 3000 UI	Dilute in NaCl 0.9% at 50 IU/ml. Aliquot it in 1 ml and store at -20°C for up to two months. Once thawed do not freeze again.
Human foreskin fibroblasts	ATCC	ATCCSCRC-1041	For long term storage it is recommended to store them in liquid nitrogen.

KnockOut Serum Replacement	Life Technologies	10828-028	Photosensitive. It is recommended to aliquot it in small volumes such as 10 ml and store it at -20°C (check the expiration date). Avoid freeze and thaw cycles.
KSOMaag Evolve	Zenith Biotech	ZEKS-050	Supplement with 4 mg/ml BSA. Equilibrate at 37°C and 5% CO ₂ a day prior to use.
Leukemia Inhibitory Factor	Merck Millipore	ESG1106	
Mice	Charles River/Harlan		It is recommended to use mice from a permissive strain in terms of mESC derivation (such as 129S2 or C57BL). However, inbred strains are less efficient producing embryos. Therefore, it is recommended to use a hybrid strain, such as 129S2 x C57BL or B6CBAF1 to take advantage of the hybrid vigor.
Mineral oil	Sigma	M8410	Embryo tested. Photosensitive.
Mitomycin C	Serva	2980501	Reconstitute the powder with MilliQ water at 0.5 mg/ml. Store at 4°C protected from light. Attention, it is harmful and suspected of causing cancer.
Mouse anti-tubulin β III (Tuj1)	Biolegend	MMS-435P	1:500 dilution
Mouse monoclonal anti- α SMA	Sigma	A5228	1:200 dilution
Mouse monoclonal anti-AFP	R&D Systems	MAB1368	1:50 dilution
Mouse monoclonal anti Oct3/4	Santa cruz	Sc-5279	1:50 dilution
Non-Essential Amino Acids	Life Technologies	11140-035	
PD0325901	Axon Medchem	1408	Reconstitute with 2.08 ml DMSO for each mg of powder, to obtain a 1 mM stock solution. Aliquot at desired volume and store at -20°C. Avoid freeze and thaw cycles.
Petri dish (35mm)	Thermo Scientific Nunc	153066	
Petri dish (60mm)	Thermo Scientific Nunc	150288	
Pregnant mare's serum gonadotropin	Foligon	Foligon-1000 UI	Dilute in NaCl 0.9% at 50 IU/ml. Aliquot it in 1 ml and store at -20°C for up to two months. Once thawed do not freeze again.
Rabbit policlonal anti-Sox2	Merck Millipore	AB5603	1:200 dilution
T75 falcon	Thermo Scientific	130190	
Trypsin-EDTA 10x	BioWest	X0930	Dilute 1:10 in HBSS to obtain a 1x solution