

Materials List for:

The Production of Pluripotent Stem Cells from Mouse Amniotic Fluid Cells Using a Transposon System

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Materials

Name	Company	Catalog Number	Comments
100 mm Bacterial-grade Petri Dishes	BD Falcon	351029	For <i>in vitro</i> differentiation
2-mercaptoethanol	Sigma	M6250	For mouse AF, iPS-AF cells and differentiation medium
Alexa568-conjugated goat anti-mouse IgM	Thermo Fisher Scientific	A21043	Secondary antibody (immunofluorescence)
Alexa594-conjugated chicken anti-goat IgG	Thermo Fisher Scientific	A21468	Secondary antibody (immunofluorescence)
Alexa594-conjugated chicken anti-rabbit IgG	Thermo Fisher Scientific	A21442	Secondary antibody (immunofluorescence)
Alexa594-conjugated goat anti-mouse IgG	Thermo Fisher Scientific	A11005	Secondary antibody (immunofluorescence)
Alkaline Phosphatase kit	Sigma	85L1	Alkaline Phosphatase staining
Ampicillin	Sigma	A0166	For bacterial selection
Bovine Serum Albumin	Sigma	A7906	BSA, for blocking solution. Diluted in PBS 1x
Chloroform	Sigma	C2432	For RNA extraction
DH5α cells	Thermo Fisher Scientific	18265-017	Bacteria for cloning procedure
Dulbecco's Modified Eagle Medium (DMEM)	Thermo Fisher Scientific	41965039	For MEF, mouse AF, iPS-AF cells and differentiation medium
Doxycycline	Sigma	D9891	For exogenous factors expression
Microcentrifuge tubes (1.5 ml)	Sarstedt	72.706	For PB production
ES FBS	Thermo Fisher Scientific	10439024	For mouse AF, iPS-AF cells and differentiation medium
FBS	Thermo Fisher Scientific	10270106	For MEF medium
Fine point forceps	F.S.T	Dumont #5	AF isolation
Gelatin	J.T.Baker	131	Used 0.1%, diluted in PBS 1x
Glycine	Bio-Rad	161-0718	For blocking solution. Diluted in PBS 1x
Haematoxylin QS	Vector Laboratories	H3404	Nuclei detection
HE	Bio-Optica	04-061010	Histological analysis of teratoma
Hoechst	Thermo Fisher Scientific	H3570	Nuclei detection
Horse Serum	Thermo Fisher Scientific	16050-122	For blocking solution
HRP-conjugated goat anti-mouse IgG	SantaCruz	sc2005	Secondary antibody (immunoperoxidase)
ImmPACT NovaRED	Vector Laboratories	SK4805	Peroxidase substrate

Insulin syringe with needle (25 G)	Terumo	SS+01H25161	Amniocentesis procedure
Klf4	SantaCruz	sc-20691	Rabbit polyclonal IgG
L-glutamine	Thermo Fisher Scientific	25030	For mouse AF, iPS-AF cells and differentiation medium
LB broth (Lennox)	Sigma	L3022	For bacterial growth
LIF	Sigma	L5158	For mouse AF and iPS-AF cells medium
Matrigel	BD	354234	For <i>in vitro</i> differentiation. Diluted 1:10 in DMEM
Methanol	Sigma	32213	Peroxidase blocking
MULTIWELL 24 well plate	BD Falcon	353047	For <i>in vitro</i> differentiation
MULTIWELL 6 well plate	BD Falcon	353046	For MEF, mouse AF and iPS-AF cells culture
Nanog	ReproCELL	RCAB0002P-F	Rabbit polyclonal IgG
Non-essential amino acids	Sigma	M7145	For mouse AF, iPS-AF cells and differentiation medium
Normal Goat Serum	Vector Laboratories	S2000	For blocking solution. Diluted in PBS 1x
NP-40	Sigma	12087-87-0	For cell permeabilization. Diluted in PBS 1x
Oct4	SantaCruz	sc-5279	Mouse monoclonal IgG2b
Oligo (dT)	Thermo Fisher Scientific	18418012	For RT-PCR
Paraformaldehyde (solution)	Sigma	441244	PFA, fixative, diluted in PBS
PBS 10x	Thermo Fisher Scientific	14200-067	D-PBS, free of Ca ²⁺ /Mg ²⁺ . Diluted with sterile water to obtain PBS 1x
Penicillin - Streptomycin	Thermo Fisher Scientific	15070063	For MEF, mouse AF, iPS-AF cells and differentiation medium
Petri Dish (150 mm)	BD Falcon	353025	For MEF culture, tissue culture
PiggyBac transposase expression plasmid	Provided by professor Andras Nagy laboratory	-	mPBBase
PiggyBac-tetO2-IRES-OKMS transposon plasmid	Provided by professor Andras Nagy laboratory	-	PB-tetO2-IRES-OKMS
QIAprep Spin Maxiprep Kit	Qiagen	12663	For plasmids purification
QIAprep Spin Miniprep Kit	Qiagen	27106	For plasmids purification
Reverse tetracycline transactivator transposon plasmid	Provided by professor Andras Nagy laboratory	-	rtTA
RNeasy Mini Kit	Qiagen	74134	For RNA extraction
Sox2	SantaCruz	sc-17320	Goat polyclonal IgG
SSEA1	Abcam	ab16285	Mouse monoclonal IgM
SuperScript II Reverse Transcriptase	Thermo Fisher Scientific	18064-014	For RT-PCR
T	Abcam	ab20680	Rabbit polyclonal IgG
Taq DNA Polymerase	Thermo Fisher Scientific	10342020	PCR
Trypsin	Thermo Fisher Scientific	25300-054	Cell culture passaging
Triton X-100	Bio-Rad	161-047	For cell permeabilization, diluted in PBS 1x
TRIzol Reagent	Thermo Fisher Scientific	15596-026	For RNA extraction
Tubb3	Promega	G712A	Mouse monoclonal IgG1

TWEEN-20	Sigma	P1379	For cell permeabilization, diluted in PBS 1x
αfp	R&D Systems	MAB1368	Mouse Monoclonal IgG1
αSMA	Abcam	ab7817	Mouse Monoclonal IgG2a
Transfection Reagent (FuGENE HD)	Promega	E2311	For AF cells transfection
Stereomicroscope	Nikon	SM2645	To perform amniocentesis
200 µl tips	Sarstedt	70.760012	To pick bacteria colonies
Scissor	F.S.T	14094-11 stainless 25U	To perform amniocentesis
Ethanol	Sigma	2860	To clean the abdominal wall of the pregnant dam
Tissue culture petri dish (150 mm)	BD Falcon	353025	For MEF expansion
Mitomycin C	Sigma	M4287-2MG	For MEF inactivation
MULTIWELL 96 well plate	BD Falcon	353071	For iPS-AF culture