Materials List for:

Serial Enrichment of Spermatogonial Stem and Progenitor Cells (SSCs) in Culture for Derivation of Long-term Adult Mouse SSC Lines

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Materials

Name	Company	Catalog Number	Comments
DMEM	Corning	10-013	Diluent for dissociation buffer
Trypsin/EDTA	Mediatech	25-051-CI	
Stem cell base medium (StemPro-34)	Life Technologies	10639-011	Requires supplementation as per Shinohara *et al*. (2003)*
Stem cell medium supplements	various	see Table 3	Requires supplementation as per Shinohara *et al*. (2003)*
JK1 cells	Cell Biolabs, Inc.	CBA-315	Can substitute with adult testicular stromal cells as per Seandel *et al*. (2007)
mitomycin-C (CAUTION)	Sigma-Aldrich	M4287	Toxic; Handle with care.
Gelatin	Sigma-Aldrich	G1890	0.4% solution in water
EVOS xl digital inverted microscope	Advanced Microscopy Group	-	
Table 1. Specific reagents and equipment. *See Table 3			
DMEM	Corning	10-013	Diluent for dissociation buffer
trypsin (1:250)	Life Technologies	27250-018	Dissociation buffer: Final 0.05% wt/vol
collagenase, type I, 235 U/ml	Worthington	CLS1 235	Dissociation buffer: Final 0.03% wt/vol
DNAse I	Sigma-Aldrich	DN25	Dissociation buffer: Final 80 U/ml
bovine serum albumin	ICP Bio	ABRE-100g	Dissociation buffer: Final 0.5% wt/vol
Table 2. Dissociation buffer			
StemPro-34 SFM	Life Technologies	10639-011	
StemPro-34 Nutrient supplement	Life Technologies	10639-011	
Additional supplements**			
Non-essential amino acids	Sigma-Aldrich	M7145	1X
MEM Vitamin solution	Life Technologies	11120-052	1X
L-glutamine	Mediatech	25-005	2 mM
bovine serum albumin	ICP Bio	ABRE	0.50%
Antibiotic-Antimycotic Solution	Mediatech	30-004-CI	1X
D(+)glucose	Sigma-Aldrich	G8769	6 mg/ml
β-estradiol	Sigma-Aldrich	E2758	30 ng/ml
progesterone	Calbiochem	5341	60 ng/ml
fetal bovine serum	variable	n/a	1%
bovine holo-transferrin	Sigma-Aldrich	T1283	100 µg/ml

insulin	Gemini Bio-Products	700-112P	25 µg/ml
human GDNF	Life Technologies	PHC7041	10 ng/ml
human bFGF	Life Technologies	PHG0023	10 ng/ml
mouse EGF	Life Technologies	PHG0313	20 ng/ml
putrescine	Research Organics	0778P	60 µM
sodium Selenite	Sigma-Aldrich	S5261	30 nM
pyruvic acid	Alfa Aesar	A13875	30 µg/ml
DL-lactic acid	J.T. Baker	0196-04	1 µg/ml
β-mercapt–thanol	Life Technologies	21985-023	50 µM
ascorbic acid	Sigma-Aldrich	A4544	100 µM
D-biotin	Sigma-Aldrich	B4639	10 µg/ml
Table 3. Stem cell medium			
***Note:** Add supplements below before using medium. Filter sterilize and keep it at 4 °C. The medium is stable for at least 2 weeks.			
**We have employed different manufacturers, formulations, and/or lot numbers of these reagents without any apparent deleterious effects. In general, cell culture grade reagents should be employed.			