CMR[B]
Centre de Medicina Regenerativa de Barcelona
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Center of Regenerative Medicine in Barcelona

CELL LINE ES[2]

Line name: ES[2]
Origin: embryonary

Data related to the biological sample:
Human embryo cryopreserved at day +6 of development (blastocyst)
Frozen: 14.06.1999
Donated: 7.03.2006
Received at the Banco de líneas celulares of CMR[B]: 9.05.2006
Thawed: 9.05.2006

General description of the process:
The cryopreserved donated embryo was thawed with by a slow protocol with PROH and sucrose. After 24h in culture, the zona pellucida (ZP) was removed by pronase. The dezoned blastocyst was cultured over a monolayer of irradiated fibroblasts in hES culture medium.

Cell and culture medium used for derivation:
Cell: human foreskin fibroblasts (ATCC, American Type Culture Collection, CCD1112Sk).
Culture medium: Knockout Dulbecco's modified Eagle's medium supplemented with 2 mmol/l GlutaMAX (Gibco, InVitrogen corporation), 0,05mmol/l 2-mercaptoethanol (Gibco, InVitrogen corporation), 8 ng/ml basic fibroblast growth factor (bFGF) (Invitrogen), 1% non-essential amino acids (Cambrex), 20% Knockout Serum Replacement (InVitrogen) y 0,5% Penicillin-Streptomycin (Gibco, InVitrogen corporation).

General description of derivation and cell line maintenance:
After 12 days in culture a cell clump appeared. It was mechanically dissociated and re-plated on a new monolayer of fibroblasts. The cells are passaged every 6-7 days. The passaging is done mechanically. Freezing of the colonies is done by a slow protocol with 90% FBS (fetal bovine serum) and 10% DMSO (dimethylsulphoxide).

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Characterization ES[2]

Code	**ES[2]**
Embryo origin	**FIV Institut Dexeus**

CHARACTERIZATION	
Passage nº	**41**
Feeders	***HUMAN FORESKIN FIBROBLASTS***
ICM isolation	**NO**
Karyotype	**46, XY**
Phenotype	
SSEA-1	**-**
SSEA-3	**+**
SSEA-4	**+**
TRA1-60	**+**
TRA1-81	**+**
***Oct* 4**	**+**
***Sox* 2**	**+**
Nanog	**+**
Alkaline Phosphatase	**+**
Freeze/thaw viability	**YES**
Pluripotency	
In vivo	**YES**
In Vitro:	
ectoderm (β-tubulin III)	**+**
endoderm (α-fetoprotein)	**+**
mesoderm (myosinE)	**+**
Microbiologic analisys	
Aerobics	**-**
Anaerobics	**-**
Fungus	**-**
Mycoplasma	**-**
HLA	**HLA-A*0101** **HLA-B*0801** **HLA-Cw*0701** **HLA-DRB1*0301** **HLA-DQB1*0201**
Fingerprinting	**Done**