

**Line name: ES[3]**  
**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).

# CELL LINE ES[3]

**Characterization ES[2]**

<b>Code</b>	<b>ES[3]</b>
<b>Embryo origin</b>	<b>FIV Institut Dexeus</b>

<b>CHARACTERIZATION</b>	
<b>Passage nº</b>	<b>20</b>
<b>Feeders</b>	<b>HUMAN FORESKIN FIBROBLASTS</b>
<b>ICM isolation</b>	<b>NO</b>
<b>Karyotype</b>	<b>46, XY</b>
<b>Phenotype</b>	
<b>SSEA-1</b>	-
<b>SSEA-3</b>	+
<b>SSEA-4</b>	+
<b>TRA1-60</b>	+
<b>TRA1-81</b>	+
<i>Oct 4</i>	+
<i>Sox 2</i>	+
<i>Nanog</i>	+
<b>Alkaline Phosphatase</b>	+
<b>Freeze/thaw viability</b>	<b>YES</b>
<b>Pluripotency</b>	
<i>In vivo</i>	<b>YES</b>
<i>In Vitro:</i>	
ectoderm ( $\beta$ -tubulin III)	+
endoderm ( $\alpha$ -fetoprotein)	+
mesoderm (myosinE)	+
<b>Microbiologic analisis</b>	
<b>Aerobics</b>	-
<b>Anaerobics</b>	-
<b>Fungus</b>	-
<i>Mycoplasma</i>	-
<b>HLA</b>	
	<b>HLA-A*0101</b>
	<b>HLA-B*0801</b>
	<b>HLA-Cw*0701</b>
	<b>HLA-DRB1*0301</b>
	<b>HLA-DQB1*0201</b>
<b>Fingerprinting</b>	<b>Done</b>