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Lab Resource: Stem Cell Line

Derivation of the clinical grade human embryonic stem cell line RCe017-A (RC-13)

P.A. De Sousa [a,b,c,*], B.J. Tye [a], K. Bruce [a], P. Dand [a], G. Russell [a], D.M. Collins [a], A. Greenshields [a], K. McDonald [a], H. Bradburn [a], A. Laurie [a], J.M. Downie [a], M. Bateman [a], A. Courtney [a]

[a] Roslin Cells Limited, Nine Edinburgh Bio-Quarter, 9 Little France Road, Edinburgh EH16 4UX, UK
[b] Centre for Clinical Brain Sciences, University of Edinburgh, UK
[c] MRC Centre for Regenerative Medicine, University of Edinburgh, UK

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ABSTRACT

The human embryonic stem cell line RCe017-A (RC-13) was derived under quality assured compliance with UK regulation, European Union Directives and International guidance for tissue procurement, processing and storage according to Good Manufacturing Practice (GMP) standards. The cell line was derived from a frozen and thawed blastocyst stage embryo voluntarily donated as unsuitable or surplus to fertility requirements following informed consent. RCe017-A (RC-13) shows normal pluripotency marker expression and differentiation to the three germ layers in vitro. It has a mixed 47XY, +12/48XY, +1, +12 male karyotype and microsatellite PCR identity, HLA and blood group typing data are available.

Resource table

Name of stem cell construct	RCe017-A
Alternative name	RC-13, RC13
Institution	Roslin Cells Ltd.
Person who created resource	B.J. Tye, K. Bruce, P. Dand, G. Russell, D.M. Collins, A. Greenshields, K. McDonald, H. Bradburn, A. Laurie
Contact person and email	Paul.desousa@roslincells.com; Paul.desousa@ed.ac.uk Janet.downie@roslincells.com Aidan.courtney@roslincells.com Malcolm.bateman@roslinfoundation.com
Date archived/stock date	11 March 2011 (seed bank)
Type of resource	Biological reagent: cell line
Sub-type	hESC, clinical grade
Origin	Blastocyst with ICM and trophoblast
Key transcription factors	Oct4 (confirmed by flow cytometry)
Authentication	See Quality Control Certificate of Analysis (Fig. 1)
Link to related literature (direct URL links and full references)	N/A
Information in public databases	http://hpscreg.eu/cell-line/RCe017-A
Ethics	Informed consent obtained. Scotland A Research Ethics committee approval obtained (07/MRE00/56). Conducted under the UK Human Fertilisation and Embryology Authority license no R0136 to centre 0202 and UK Human Tissue Authority (HTA) licensing number 22631.

Resource details

RCe017-A (RC-13) was derived from a frozen and thawed blastocyst stage embryo that was surplus to requirement or unsuitable for clinical use. Human embryonic stem cell (hESC) isolation, expansion and qualification were performed in facilities whose specification, operation and monitoring complied with GMP standards enabling; i) a fully traceable procurement procedure with informed ethical consent which includes provision for commercial use, ii) detailed medical history and blood borne virus (BBV) screening of donors, and iii) compilation of a cell line history providing details on hESC manufacturing process and quality control testing regime.

Human ESC culture and processing were performed in a grade A tissue culture cabinet in a grade B clean room environment monitored for particulate and microbiological contamination during cell processing in accordance with Rules and Guidance for Pharmaceutical Manufacturers and Distributors — The Orange Guide, compiled by the UK Medicines Healthcare Products Regulatory Authority (go to: https://www.gov.uk/guidance/good-manufacturing-practice-and-good-distribution-practice). Accordingly, the facility was operating under a mature Quality Management System, compliant with

* Corresponding author at: Paul De Sousa PhD MSc Reader Centres for Clinical Brain Sciences & Regenerative Medicine, University of Edinburgh, Chancellor's Building, 49 Little France Crescent, Edinburgh, EH16 4SB Scotland, UK Tel: +44 (0)-131-242-6646 E-mail: paul.desousa@ed.ac.uk Web: www.crm.ed.ac.uk/research/associate/pluripotent-cell-translation Chief Scientist, Executive Director, Founder Roslin Cells Ltd. Scottish Centre for Regenerative Medicine, 5 Little France Drive, Edinburgh, EH16 4UU Scotland, UK Tel: +44(0)7917594876 E-mail: paul.desousa@roslincells.com Web: www.roslincells.com
E-mail address: paul.desousa@ed.ac.uk (P.A. De Sousa).

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ISO9001:2008 standards. hESC derivation was performed under licensure from the UK HFEA (R0136 to centre 0202) and HTA (Licensing Number 22631).

hESC derivation involved whole embryo outgrowth on mitotically inactivated human dermal fibroblast (HDF) feeder cells. HDFs were derived and manufactured according to GMP and had been approved for clinical use by the Food and Drug Administration, USA. During derivation on HDFs, hESCs were grown in a xeno-free cell therapy grade media (XF KODMEM) supplemented with xeno-free human recombinant bFGF. hESCs were subsequently expanded in a GMP grade serum-free medium (StemPro hESC Serum Free Medium) on a xeno-free matrix (CellStart). The former contained bovine serum albumin (BSA) from a Transmissible Spongiform Encephalopathy (TSE)-free country of origin. The cell line was cryopreserved in a GMP compliant cryopreservation solution (CryoStor CS10).

By flow cytometry, RCe017-A (RC-13) expressed the pluripotency makers Oct-4, Tra-1-60 and SSEA-4 (80.8%, 88.3% and 79.6%, respectively), whereas low expression of the differentiation marker SSEA-1 (0.7%) was observed (Figs. 1 and 2). Differentiation to the three germ layers, endoderm, ectoderm and mesoderm, was demonstrated using embryoid body formation in vitro, and expression of the germ layer markers α-fetoprotein, β-tubulin and muscle actin was observed (Fig. 3).

A microsatellite PCR profile has been obtained for the cell line, and HLA Class I and II typing is available (Table 1). Blood group genotyping gave the blood group AA (Table 1).

Verification and authentication

The cell line was analysed for genome stability by G-banding and showed a mixed male genotype. Trisomy 12 (47XY, +12) was present in 26 of 30 cells analysed, but a subpopulation (4 of 30 cells) has an additional copy of chromosome 1 (48XY, +1, +12) (Fig. 4). The cell line is free from mycoplasma contamination as determined by RT-qPCR.

ROSLIN CELLS

Quality Control Test Certificate

Sample Point 2 Test Results

Certificate Number:	QCC-11-257	**Version:**	1
Grade:	CLINICAL		
Sample ID:	RC-13 P19A		

Assay	Test Method	Roslin Cells Assay Code	Date of Assay	Result
Mycoplasma Detection	RT-qPCR (SOP/QCP/22)	MYCO-11-004	13Apr11	**Not Detected****
Endotoxin Detection	Kinetic Chromogenic LAL SOP/QCP/12)	ENDO-10-007	09Dec10	**1.04 EU/ml**
Viral Screening*	PCR (CMV,HTLV1,HIV1,HCV, HBV,EBV) (SOP/QCP/60)	N/A	10Dec10	**Not Detected**
Karyotype*	G-banding (SOP/QCP/59)	N/A	08May11	**47,XY,+12(26)/48,XY,+1,+12(4)*****
Pluripotency / Differentiation	Flow Cytometry (SOP/QCP/25)	FLOW-10-008	09Dec10	**% Positive** **SSEA-4 – 79.6** **Oct 3/4 – 80.8** **Tra-1-60 – 88.3** **SSEA-1 – 0.7**
Microsatellite Genotyping*	PCR (SOP/QCP/6)	N/A	19Apr11	**ID Obtained**

*Subcontracted to a Third Party
**Analysis performed on RC-13 P27A
***Analysis performed on RC-13 P26A

Certificate Prepared by (QC): [signature] Date: 060CT11

Certificate Reviewed by (QC): Audrey Laurie Date: 11OCT11.

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Fig. 1. Quality Control Certificate of Analysis for RCe017-A (RC-13) P19A seed lot.