Stem Cell Research 16 (2016) 751–755

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Derivation of the clinical grade human embryonic stem cell line RCe019-A (RC-15)

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ARTICLE INFO

Article history:
Received 28 March 2016
Accepted 5 April 2016
Available online 13 April 2016

ABSTRACT

The human embryonic stem cell line RCe019-A (RC-15) 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 cleavage stage embryo voluntarily donated as unsuitable or surplus to fertility requirements following informed consent. RCe019-A (RC-15) shows normal pluripotency marker expression and differentiation to the three germ layers in vitro. It has a mixed 46XX/47XX, +8 female karyotype and microsatellite PCR identity, HLA and blood group typing data is available.

Resource table.

Name of stem cell construct	RCe019-A
Alternative name	RC-15, RC15
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	20 April 2011 (seed bank)
Type of resource	Biological reagent: cell line
Sub-type	hESC, clinical grade
Origin	Cleavage stage embryo (Mitosis)
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/RCe019-A
Ethics	Informed consent obtained. Scotland A Research Ethics committee approval obtained (07/MRE00/56). Conducted under the UK Human Fertilisation and Embryology Authority licence no R0136 to centre 0202 and UK Human Tissue Authority (HTA) licensing number 22631.

1. Resource details

RCe019-A (RC-15) was derived from a 3–4 cell cleavage stage embryo that was surplus to requirement or unsuitable for clinical use and cultivated to the blastocyst stage. 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 ISO9001:2008 standards. HESC derivation was performed under licensure from the UK HFEA (R0136 to centre 0202) and HTA (Licensing Number 22631).

http://dx.doi.org/10.1016/j.scr.2016.04.003

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, RCe019-A (RC-15) expressed the pluripotency makers Oct-4, Tra-1-60 and SSEA-4 (87.4%, 80.7% and 94.6%, respectively), whereas low expression of the differentiation marker SSEA-1 (2.4%) was observed (Fig. 1, Fig. 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 O_1O_1 (Table 1).

1.1. Verification and authentication

The cell line was analysed for genome stability by G-banding and showed a mixed female genotype. A normal 46XX genotype was present in 21 of 30 cells analysed, but an extra copy of chromosome 8 (47XX, +8) was present in a subpopulation (8 of 30 cells) of cells (Fig. 4). The cell line is free from mycoplasma contamination as determined by RT-qPCR.

2. Materials and methods

2.1. Ethics

Derivation of hESC from surplus to requirement and failed to fertilise/develop embryos was approved by The Scotland A Research Ethics Committee and local ethics board at participating fertility clinics

Roslin Cells

Quality Control Test Certificate

Sample Point 2 Test Results

Certificate Number:	QCC-11-213	**Version:**	1
Grade:	CLINICAL		
Sample ID:	RC-15 P24B CS		

Assay	Test Method	Roslin Cells Assay Code	Date of Assay	Result	
Mycoplasma Detection	RT-qPCR (SOP/QCP/22)	MYCO-11-004	13 Apr 11	**Not Detected**	
Endotoxin Detection	Kinetic Chromogenic LAL (SOP/QCP/12)	ENDO-11-008	18 Apr 11	**1.39 EU/ml**	
Viral Screening*	PCR (CMV,HTLV1,HIV1,HCV, HBV,EBV) (SOP/QCP/60)	N/A	21 Apr 11	**Not Detected**	
Karyotype*	G-banding (SOP/QCP/51)	N/A	18 Apr 11	**47, XX, +8(8)/46,XX(21)**	
Pluripotency / Differentiation	Flow Cytometry (SOP/QCP/25)	FLOW-11-005	20 Apr 11	**Antibody**	**% Positive**
				SSEA-4	**94.6**
				Oct 3/4	**87.4**
				Tra-1-60	**80.7**
				SSEA-1	**2.4**
Microsatellite Genotyping*	PCR (SOP/QCP/6)	MPCR-11-001	02 Aug 11	**ID Obtained**	

*Subcontracted to a Third Party

Certificate Prepared by (QC): E. Clarke Date: 30 NOV 11

Certificate Reviewed by (QC): [signature] Date: 30nov11

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Fig. 1. Quality Control Certificate of Analysis for RCe019-A (RC-15) P24B seed lot.