Stem Cell Research 17 (2016) 36–41

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 RCe013-A (RC-9)

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], M.A. Canham [c], T. Kunath [c], 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

ARTICLE INFO

Article history:
Received 22 April 2016
Accepted 28 April 2016
Available online 6 May 2016

ABSTRACT

The human embryonic stem cell line RCe013-A (RC-9) 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 failed to fertilise oocyte voluntarily donated as unsuitable and surplus to fertility requirements following informed consent. RCe013-A (RC-9) shows normal pluripotency marker expression and differentiation to the three germ layers in vitro and in vivo. It has a normal 46XY male karyotype and microsatellite PCR identity, HLA and blood group typing data are available.

Resource table

Name of stem cell construct	RCe013-A
Alternative name	RC-9, RC9
Institution	Roslin Cells Ltd.
Person who created resource	B. Tye, K. Bruce, P. Dand, G. Russell, D.M. Collins, A. Greenshields, J. Marshall, K. McDonald, H. Bradburn, M.A. Canham
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 Tilo.kunath@ed.ac.uk
Date archived/stock date	10 September 2014 (Seed lot, Passage 28)
Type of resource	Biological reagent: cell line
Sub-type	hESC, clinical grade
Origin	Zygote (Oocyte/1PN)
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/RCe013-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.

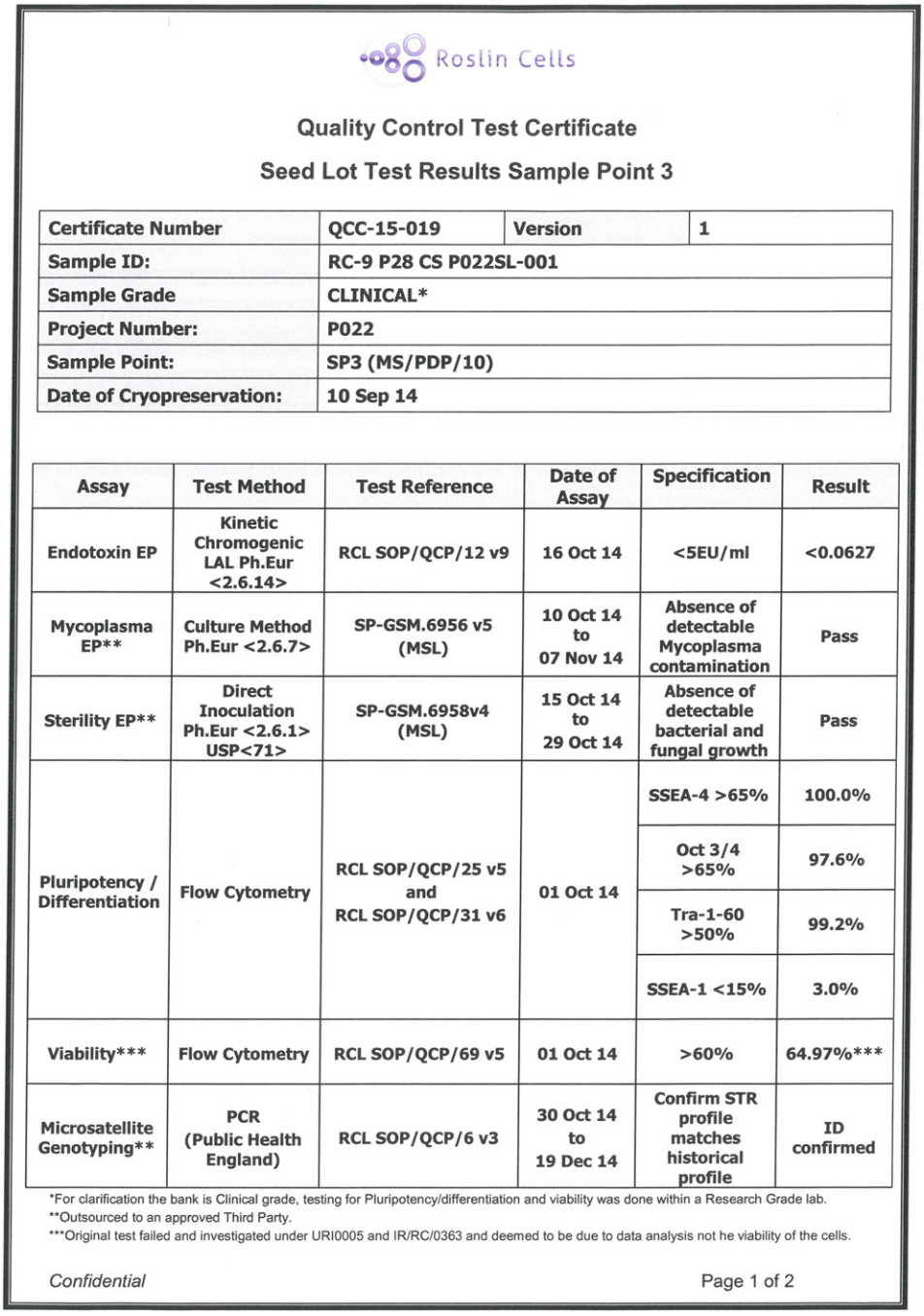
Resource details

RCe013-A (RC-9) was received as a failed to fertilise oocyte/1PN (pro-nuclear) embryo that was surplus to requirement or unsuitable for clinical use due to late development. Human embryonic stem cell (hESC) isolation, expansion and qualification was performed in a 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 was 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. Further hESC derivation was performed under licensure from the UK HFEA (R0136 to centre 0202) and HTA (Licensing Number 22631).

The embryo was grown to blastocyst stage and the cell line was derived by 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

http://dx.doi.org/10.1016/j.scr.2016.04.020



Roslin Cells

Quality Control Test Certificate

Seed Lot Test Results Sample Point 3

Certificate Number	**QCC-15-019**	**Version**	**1**
Sample ID:	**RC-9 P28 CS P022SL-001**		
Sample Grade	**CLINICAL***		
Project Number:	**P022**		
Sample Point:	**SP3 (MS/PDP/10)**		
Date of Cryopreservation:	**10 Sep 14**		

Assay	Test Method	Test Reference	Date of Assay	Specification	Result
Endotoxin EP	Kinetic Chromogenic LAL Ph.Eur <2.6.14>	RCL SOP/QCP/12 v9	16 Oct 14	<5EU/ml	<0.0627
Mycoplasma EP**	Culture Method Ph.Eur <2.6.7>	SP-GSM.6956 v5 (MSL)	10 Oct 14 to 07 Nov 14	Absence of detectable Mycoplasma contamination	Pass
Sterility EP**	Direct Inoculation Ph.Eur <2.6.1> USP<71>	SP-GSM.6958v4 (MSL)	15 Oct 14 to 29 Oct 14	Absence of detectable bacterial and fungal growth	Pass
Pluripotency / Differentiation	Flow Cytometry	RCL SOP/QCP/25 v5 and RCL SOP/QCP/31 v6	01 Oct 14	SSEA-4 >65%	100.0%
				Oct 3/4 >65%	97.6%
				Tra-1-60 >50%	99.2%
				SSEA-1 <15%	3.0%
Viability***	Flow Cytometry	RCL SOP/QCP/69 v5	01 Oct 14	>60%	64.97%***
Microsatellite Genotyping**	PCR (Public Health England)	RCL SOP/QCP/6 v3	30 Oct 14 to 19 Dec 14	Confirm STR profile matches historical profile	ID confirmed

*For clarification the bank is Clinical grade, testing for Pluripotency/differentiation and viability was done within a Research Grade lab.
**Outsourced to an approved Third Party.
***Original test failed and investigated under URI0005 and IR/RC/0363 and deemed to be due to data analysis not he viability of the cells.

Confidential Page 1 of 2

Fig. 1. Quality Control Certificate of Analysis for RCe013-A (RC-9) P28 seed lot.

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,) and xeno-free matrix (CellStart). The former contained bovine serum albumin (BSA) from a Transmissible Spongioform Encephalopathy (TSE)-free country of origin. The cell line was cryopreserved in a GMP compliant cryopreservation solution (CryoStor CS10).

By flow cytometry, RCe013-A (RC-9) expressed the pluripotency makers Oct-4, Tra-1-60 and SSEA-4 (98.9%, 99.2% and 100.0%, respectively), whereas low expression of the differentiation marker SSEA-1 (3.0%) 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, top panel). In vivo teratoma formation yielded typical hESC derived teratomas. Histological examination of fixed and stained sections clearly showed generation of cell types from ectoderm and mesoderm lineages. Endoderm differentiation was also present, but the quality of the structures meant these could not be fully characterised (Fig. 3, bottom panels).

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 BB (Table 1). The cell line is free from mycoplasma contamination as determined by RT-qPCR.