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# Derivation of the clinical grade human embryonic stem cell line RCe020-a (RC-16)

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ABSTRACT

The human embryonic stem cell line RCe020-A (RC-16) 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 or surplus to fertility requirements following informed consent. RCe020-A (RC-16) shows normal pluripotency marker expression and differentiates to mesoderm and potentially ectoderm in vitro. It has an abnormal 47XX, + 14, i(20)(q10) female karyotype and microsatellite PCR identity, HLA and blood group typing data is available.



## 1. Resource table

| | |
|---|---|
| Name of stem cell construct | RCe020-A |
| Alternative name | RC-16, RC16 |
| 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 | 23 March 2011 (seed bank) |
| 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/RCe020-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. |

## 2. Resource details

RCe020-A (RC-16) was received as a failed to fertilise oocyte/1PN (pro-nuclear) embryo that was surplus to requirement or unsuitable for clinical use and was cultivated to blastocyst stage. 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 anufacturers 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).

Derivation of hESCs 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.

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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 Spongioform Encephalopathy (TSE)-free country of origin. The cell line was cryopreserved in a GMP compliant cryopreservation solution (CryoStor CS10).

By flow cytometry, RCe020-A (RC-16) expressed the pluripotency makers Oct-4, Tra-1-60 and SSEA-4 (85.7%, 85.8% and 94.6%, respectively), whereas low expression of the differentiation marker SSEA-1 (0.0%) was observed (Figs. 1, 2). To assess the differentiation potential of the cell line, embryoid body formation in vitro was used and showed differentiation to the mesoderm lineage by expression of muscle actin. RCe020-A (RC-16) may also be able to differentiate to ectoderm, as atypical staining with an antibody to β-tubulin was also observed, but at the seed banking stage there was no expression of α-fetoprotein (endoderm) (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 $AO_1$ (Table 1).

## 3. Verification and authentication

The cell line was analysed for genome stability by G-banding and showed a female genotype with extra copy of chromosome 14, in addition to an isochromosome for the long arm of chromosome 20 (47XX, +14, i(20)(q10)) in all 30 cells examined (Fig. 4). The cell line is free from mycoplasma contamination as determined by RT-qPCR.

## 4. Materials and methods

### 4.1. Ethics

Derivation of hESC from surplus to requirement and failed to fertilise/develop embryos was approved by The Scotland A

**ROSLIN CELLS**

**Quality Control Test Certificate**

**Sample Point 2 Test Results**

| | | | |
|---|---|---|---|
| **Certificate Number:** | QCC-11-182 | **Version:** | 1 |
| **Grade:** | CLINICAL | | |
| **Sample ID:** | RC-16 P18A CS | | |

| Assay | Test Method | Roslin Cells Assay Code | Date of Assay | Result |
|---|---|---|---|---|
| Mycoplasma Detection | RT-qPCR (SOP/QCP/22) | MYCO-11-003 | 24Mar11 | **Not Detected** |
| Endotoxin Detection | Kinetic Chromogenic LAL (SOP/QCP/12) | ENDO-11-007 | 24Mar11 | **1.54 EU/ml** |
| Viral Screening* | PCR (CMV,HTLV1,HIV1,HCV, HBV,EBV) (SOP/QCP/60) | N/A | 24Mar11 | **Not Detected** |
| Karyotype* | G-banding (SOP/QCP/59) | N/A | 11May11 | **47,XX,+14,i(20)(q10)**** |
| Pluripotency / Differentiation | Flow Cytometry (SOP/QCP/25) | FLOW-11-004 | 23Mar11 | **% Positive**<br>**SSEA-4 – 94.9**<br>**Oct 3/4 – 85.7**<br>**Tra-1-60 – 85.8**<br>**SSEA-1 – 0** |
| Microsatellite Genotyping* | PCR (SOP/QCP/6) | N/A | 19Apr11<br>10May11 | **ID Obtained***** |

*Subcontracted to a Third Party
**Analysis performed on RC-16 P22A
***Analysis repeated on 10May11 due to failure of one of triplicates on 19Apr11

Certificate Prepared by (QC): _____ Date: 12OCT11

Certificate Reviewed by (QC): Audrey Laure Date: 12OCT11

*Confidential*

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**Fig. 1.** Quality Control Certificate of Analysis for RCe020-A (RC-16) P18A seed lot.