CR4 Human Colorectal Cancer Cell Line

Cancer Cell Line

Cat. # SCC107

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Certificate of Analysis

Background

Colorectal cancer (CRC) is the 2nd highest cause of cancer death in the United States. Increasing evidence suggests that a small population of cancer-initiating cells (CICs) or cancer stem cells (CSCs) are responsible for tumorigenesis and the exceptional drug resistance to anti-cancer therapies.

CR4 is a novel CIC-enriched, highly tumorigenic, clonogenic and 3D spheroid-forming colon cancer cell line derived from the liver metastasis of a male patient with stage 4 colon cancer[1]. Primary tumor cells were established on type I collagen coated flasks in serum-free stem cell media[1]. Holoclones, small stem-like cells that are densely packed, were selectively subcloned from the heterogeneous culture and propagated. Cells were then passaged as 3D spheroids and in NOD/SCID mice xenografts leading to the eventual establishment of the CR4 human colorectal cancer cell line.

Majority of CR4 cells express high but variable levels of common cancer stem cell markers including CD44, CD166, EpCAM, and Lgr5[1]. CR4 cell line has the G12V mutation on KRAS exon 2 (Botchkina GI, communications).

Short Tandem Repeat (STR) Profile

D3S1358: 14	D16S539: 12
TH01: 6, 9	CSF1PO: 10, 11
D21S11: 28, 32.2	Penta D: 8, 11
D18S51: 19	vWA: 17, 19
Penta E: 13	D8S1179: 10, 13
D5S818: 12	TPOX: 8
D13S317: 12	FGA: 22
D7S820: 8, 9	Amelogenin: X

Cancer cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Storage and Handling

CR4 Human Colorectal Cancer Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

References

1. Rowehl RA, Burke S, Bialkowska AB, Pettet DW III, Rowehl L, Li E, Antoniou E, Zhang Y, Bergamaschi R, Shroyer KR, Ojima I., Botchkina GI (2014) *PLOS* 9(6): e99091.

Quality Control Testing

- Each vial contains ≥ 1X10^6 viable cells.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, B, C, Herpesvirus type 7, 8 and HIV-1 & 2 viruses by PCR
- **Cells are positive for herpesvirus type 6 by PCR analysis.**
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Representative Data

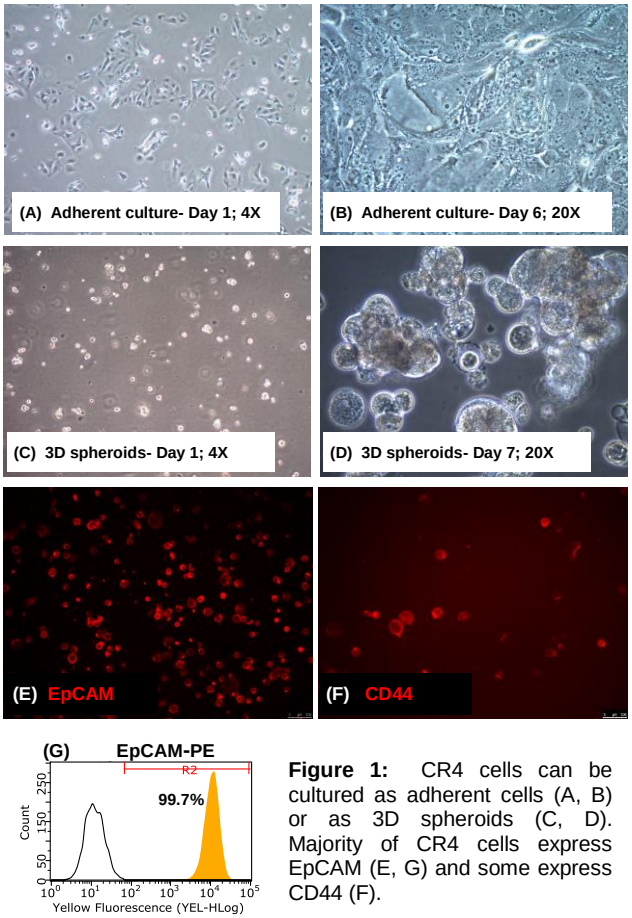

Figure 1: CR4 cells can be cultured as adherent cells (A, B) or as 3D spheroids (C, D). Majority of CR4 cells express EpCAM (E, G) and some express CD44 (F).

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Protocols

CR4 cells will tend to form tightly knit clusters (an indication of cancer stem cell activity) that are difficult to dissociate into single cells at passage. CR4 cells proliferate relatively slowly. Upon thawing a vial into a collagen I coated T75 flask, it will take approximately 6 days for cells to be ~80-85% confluent and ready to be passaged. To maintain their stemness, seed CR4 cells at low density. Monitor the culture daily to ensure that cells are not overgrown or reach full confluency. CR4 cells are mostly small (~5-7 μm) but cultures may contain a few rare very large (often 200 μm) gigantic, multinucleated cells as well. The best criterion of the retained "stemness" is the induction of roundish colonies (holoclones) of small, densely packed cells.

CR4 Expansion Medium

CR4 may be cultured in PluriSTEM ES/iPS Expansion Medium (MilliporeSigma Cat. No. SCM130) **OR** TheraPEAK™ Chemically Defined Mesenchymal Stem Cell Growth Medium MSCGM-CD (Lonza Cat. No. 00190632).

Use of Collagen I for adherent and 3D spheroid cultures

CR4 may be cultured as adherent cells on Collagen I coated flasks (BD BioCoat Cellware, Fisher Cat. No. 08774337) or as 3D spheroids in ultra-low adhesion flasks in CR4 expansion medium containing 20 μg/mL collagen I. For adherent cultures, we recommend use of the pre-coated flasks. Other pre-coated flasks have not been tested. It is a good idea to culture CR4 cells periodically in 3D culture systems to ensure continued maintenance of stem cell characteristics.

Note: *CR4 cells are strongly adherent on Collagen I coated flasks and will require 20-30 minutes incubation with Accutase or trypsin to detach the cells.*

Thawing Cells

1. Do not thaw the cells until the recommended medium and collagen I coated flasks are on hand.

   Note: Cells are thawed in CR4 Expansion Medium supplemented with 10% FBS. After thawing, cells should be cultured in CR4 Expansion Medium alone (without FBS supplementation).
2. Remove the vial of frozen CR4 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. **IMPORTANT: Do not vortex the cells.**
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of CR4 Expansion Medium supplemented with 10% FBS (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
8. Resuspend the cells in 10-15 mL of CR4 Expansion Medium (without FBS supplementation). Transfer the cell mixture to a collagen I coated T75 tissue culture flask.
9. Incubate the cells at 37°C in a humidified incubator with 5% CO_2. The next day, exchange the medium with 10-15 mL of fresh CR4 Expansion Medium. Exchange with fresh medium every two to three days thereafter.
10. When the cells are approximately 80-85% confluent, they can be dissociated with Accutase (EMD Millipore Cat. No. SCR005) or trypsin-EDTA (EMD Millipore Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use.

Subculturing Cells

1. Carefully remove the medium from the collagen I coated T75 tissue culture flask containing the 80-85% confluent layer of CR4 cells.
2. Rinse the T75 flask twice with 10 mL 1X PBS. Aspirate after each rinse. Apply 3-5 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for **20-30** minutes.
3. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
4. Add 10 mL of CR4 Expansion Medium to the flask. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
5. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
6. Apply 2 mL of CR4 Expansion Medium to the conical tube and resuspend the cells thoroughly. **IMPORTANT: Do not vortex the cells.**
7. Count the number of cells using a hemocytometer.
8. Plate the cells to the desired density. Typical split ratio is 1:6 to 1:10.

   For adherent cultures: Passage CR4 cells into collagen I coated flasks.

   For 3D spheroid cultures: Passage CR4 cells into ultra-low attachment flasks in CR4 expansion medium containing 20 μg/mL collagen I. For subsequent passages, gently disaggregate spheroids by repeated pipetting and transfer into ultra-low-attachment flasks.

Cryopreservation of Cells

CR4 Human Prostate Cancer Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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