

A Three-dimensional Model of Spheroids to Study Colon Cancer Stem Cells

 Maria Virginia Giolito^{1,2}, Léo Claret^{1,2}, Carla Frau¹, Michelina Plateroti^{1,2}

¹Département de la recherche, Centre de Recherche en Cancérologie de Lyon, INSERM U1052, CNRS UMR5286, Université de Lyon, Université Lyon 1, Centre Léon Bérard ²UMR-S1113 - IRFAC INSERM, Université de Strasbourg

Corresponding Author

Michelina Plateroti
plateroti@unistra.fr

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Materials

| Name | Company | Catalog Number | Comments |
|--|--|----------------|--|
| 37 µm Reversible Strainer, Large | STEMCELL Technologies | 27250 | To be used with 50 mL conical tubes |
| 5-Fluorouracil | Gift from Pharmacy of the Centre Leon Berard (CLB) | - | stock solution, 5 mg/100 mL; final concentration, 50 µg/mL |
| Agarose | Sigma | A9539 | |
| Aggrewell 400 24-well plates | STEMCELL Technologies | 34411 | 1,200 microwells per well for spheroid formation and growth |
| Anti Caspase3 - Rabbit | Cell Signaling | 9661 | dilution 1:200 |
| Anti Musashi-1 (14H1) - Rat | eBioscience/Thermo Fisher | 14-9896-82 | dilution 1:500 |
| Anti-Adherence Rinsing Solution x 100 mL | STEMCELL Technologies | 07010 | |
| Anti-CD133 (13A4) - Rat | Invitrogen | 14-133-82 | dilution 1:100 |
| Anti-CD44 -Rabbit | Abcam | ab157107 | dilution 1:2000 |
| Anti-PCNA - Mouse | Dako | M0879 | dilution 1:1000 |
| Anti-β-catenin - Mouse | Santa Cruz Biotechnology | sc-7963 | dilution 1:50 |
| Black multiwell plates | Thermo Fisher Scientific | 237108 | |
| Citric Acid Monohydrate | Sigma | C1909 | |
| CLARIOstar apparatus | BMG Labtech | | microplate reader |
| Dako pen | | | marker pen to mark circles on slides for creating barriers for liquids |
| Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 488 | Thermo Fisher Scientific | A21202 | dilution 1:1000 |
| Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 568 | Thermo Fisher Scientific | A10037 | dilution 1:1000 |
| Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | Thermo Fisher Scientific | A21206 | dilution 1:1000 |
| Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 | Thermo Fisher Scientific | A10042 | dilution 1:1000 |

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|---|--------------------------|-------------|---|
| Dulbecco's Modified Eagle Medium (DMEM) Glutamax (L-alanyl-L-glutamine dipeptide) | Gibco | 10569010 | |
| Fetal Bovine Serum (FBS) | Gibco | 16000044 | |
| Fluorogel mounting medium with DAPI | Interchim | FP-DT094B | |
| Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 | Thermo Fisher Scientific | A11077 | dilution 1:1000 |
| ImageJ software | | | Spheroid image analysis |
| Irinotecan | Gift from Pharmacy CLB | - | stock solution, 20 mg/mL; final concentration, 100 µg/mL |
| iScript reverse transcriptase | Bio-Rad | 1708891 | |
| Leucovorin | Gift from Pharmacy CLB | - | stock solution, 50 mg/mL; final concentration, 25 µg/mL |
| Matrigel Basement Membrane Matrix | Corning | 354234 | Basement membrane matrix |
| Nucleospin RNA XS Kit | Macherey-Nagel | 740902 .250 | |
| Oxaliplatin | Gift from Pharmacy CLB | - | stock solution, 100 mg/20 mL; final concentration, 10 µg/mL |
| Penicillin-streptomycin | Gibco | 15140130 | |
| Phosphate Buffer Saline (PBS) | Gibco | 14190250 | |
| SYBR qPCR Premix Ex Taq II (Tli RNaseH Plus) | Takara | RR420B | |
| SYTOX- Green | Thermo Fisher Scientific | S7020 | nucleic acid stain; dilution 1:5000 |
| Trypsin-EDTA (0.05 %) | Gibco | 25300062 | |
| Zeiss-Axiovert microscope | | | inverted microscope for acquiring images of spheroids |