

CH22

Introduction

The CH22 cell line was developed by Francis Hornicek and Zhenfeng Duan at Massachusetts General Hospital from a peritoneum metastatic lesion presenting in a 56 year old female with sacral chordoma. It is one of the fastest growing and easier to maintain chordoma cell lines.

Media formulation

Materials

- IMDM (Gibco, Cat# 12440079)
- RPMI 1640 (Gibco, Cat# 11875085)
- FBS, Certified, US (Gibco, Cat# 16000044) or equivalent quality FBS
- MEM NEAA (Corning, Cat# 25-025-CIR)
- GlutaMAX (Gibco, Cat#35050061)
- Nalgene Rapid-Flow Vacuum Filter (Thermo Scientific, Cat# 5660010)

(4:1) IMDM/RPMI 1640 + 10% FBS + 1% NEAA + 1% GlutaMAX media preparation protocol

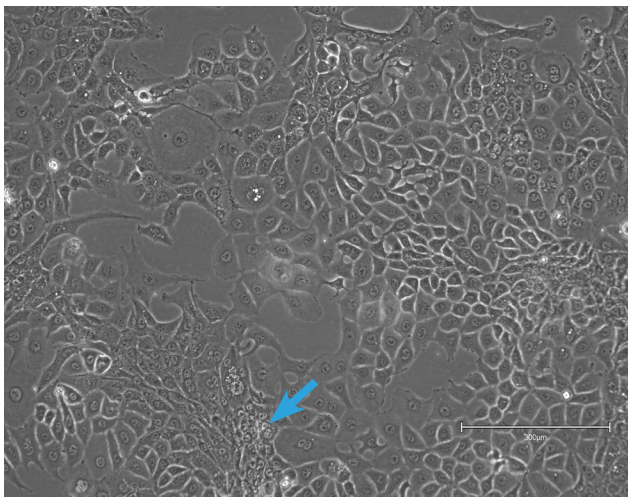
1. Prepare media in the bio-safety cabinet using aseptic technique. This media is made without antibiotics so extra precautions need to be taken.
2. Douse items in 70% Ethanol and place in bio-safety cabinet. Wait at least 10 minutes so ethanol has a chance to disinfect. Check for liquid at base of cap, if present, aspirate.
3. Create 500mL of media using volumes to the right by pipetting the media components into the upper compartment of a 500mL Nalgene vacuum filter, filter the components, and then store media at 4°C.

Component	Volume (mL)
IMDM	352
RPMI	88
FBS	50
NEAA	5
Glutamax	5
Total	500

Culturing characteristics

This cell line is thawed using CF Labs general thawing protocol, [found here](#). This line can be grown on standard tissue culture treated, non-collagen coated vessel surfaces as this line's viability and growth are not dependent on collagen. Using the above media formulation and grown in the absence of collagen, CH22 cells exhibit a doubling time of approximately two days. The cell line exhibits a heterogeneous physaliferous morphology. **(A)** CH22 representative image. The cells will grow to form tightly packed clusters, as indicated by the blue arrows in the image below. When culturing, it is important to monitor the health of the cells at the center of the aggregate, as they may begin to die as the aggregate density increases. **(B)** In a 96-well plate, plating 1,000 cells/well (3,125 cells/cm²) will yield 15% confluence 36 hours after plating, labeled as day 0 where 36 hours is the time allowed for cells to adhere and spread out on the plate. The cells reach 90% confluence after five days. This information can be extrapolated to other vessel types.

A.



B.

