

FRT flp CFTR cell culture

Medium:

- Ham's F-12, Coon's Modification (Sigma, cat. no. F6636) with 5% FBS.

Preparation Instructions

http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Product_Information_Sheet/1/f6636pis.Par.0001.File.tmp/f6636pis.pdf

Powdered media is hygroscopic and should be protected from moisture. The entire contents of each package should be used after opening. Preparing a concentrated solution of medium is not recommended as precipitates may form. Supplements can be added prior to filtration or introduced aseptically.

1. Measure 90% of final required volume of water. Water temperature should be 15-20 °C.
2. While gently stirring the water, add powdered medium. Stir until dissolved. Do NOT heat.
3. Rinse original package with a small amount of water to remove all traces of powder. Add to solution in step 2.
4. To the solution in step 3, add 2.68 g sodium bicarbonate or 35.7 ml of sodium bicarbonate solution [7.5% w/v] for each liter of medium being prepared. Stir until dissolved.
5. While stirring, adjust pH of the medium to 0.1-0.3 pH units below the desired pH (since pH may rise during filtration). The use of 1N HCl or 1N NaOH is recommended to adjust pH.
6. Add additional water to bring the solution to final volume.
7. Sterilize immediately by filtration using a membrane with porosity of 0.22 microns.
8. Aseptically dispense medium into sterile container.
9. Add FBS to 5%.

Chemicals:

- Zeocin (Thermo Fisher, R250-1). Use at 100-200 µg/ml for FRT flp4 base cells (these cells were initially established by selection using 600 µg/ml).
- Hygromycin (Thermo Fisher, 10687010). Use at 100 µg/ml for FRT flp-CFTR cells.
Caution: Check active hygromycin content in the stock. Hygromycin from Thermo Fisher is ~90% active. Purity can vary depending on the source and working concentration may need to be adjusted accordingly.

Cell culture:

- Change the medium 2-3 times per week.
- To split cells, use trypsin-EDTA and PBS.
- The cells have a doubling time of approximately 24 hours.

For More Information:

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