

Media Preparation Protocol

Fredberg Lab

21-02-2016

1. General comments regarding ingredients

Gather media components and thaw if frozen:

DMEM and EMEM are stored in bulk in cold room.

Do not freeze DMEM/EMEM. Do not keep at 37°C for prolonged periods. Use the **correct DMEM/EMEM** for the appropriate media – see recipes.

Aliquoted FBS is stored in freezer C. Thaw at room temperature. Thawing in bath is not recommended as it might damage the FBS. If possible, thaw overnight in 4°C refrigerator.

Pen-Strep is stored in freezer A. Thaw in 37°C water bath.

2. MDCK Media recipe

Attached are links to ATCC's [MDCK cell line](#), [EMEM](#), [FBS](#) and [Pen-strep](#). The recipe can be found at ATCC's MDCK cell [culture method](#). Note, they do not use Penstrep.

- EMEM 1x (Eagle's Minimum Essential Medium):
- 10% FBS (Fetal Bovine Serum)
- 1% Pen-Strep (Penicillin Streptomycin)

1. Bring sterilized filter bottle into hood. Open packaging under hood. Ensure that the small connector to the vacuum tube remains undamaged
2. Prepare media in culture hood:
 - Pour 500 ml DMEM into the top compartment of the filter bottle.
 - Pipette 50 ml of FBS into filter.
 - Pipette 5 ml of Pen-Strep into filter.

Important – once the filter has been wetted, the clock is ticking. Just make sure to pour in all ingredients while the filter is wetted. So long as aspiration has not started you have plenty of time.

3. Apply suction to speed up the fluid filtration.
4. Once all ingredients are filtered, cap bottle and swirl to mix.
5. Label flask with ingredients, date, media name and your name.
6. If needed immediately place in 37°C bath else store media in 4°C.
7. Monitor media color. Reddish color is good. If the liquid is pinkish, then the pH is high. Dispose of the media and prepare fresh batch.