

What medium should be used for culturing fibroblasts?

See the Catalog at <https://www.coriell.org/> for details of the culture medium for individual cell lines in the Culture Protocol tab of the individual sample details page.

We add L-glutamine (or equivalent) to a final concentration of 2 mM just before use. If long-term storage of cell culture medium is not an issue, commercially prepared medium containing L-glutamine (or equivalent) can be used.

Coriell Institute does not use antibiotics or antifungals because of the danger of a cryptic infection in a cell repository. An investigator can add antibiotics if desired.

If a cell culture is growing slower than expected, our first approach is to switch to a different lot of pre-tested serum and/or to alter the serum concentration by 5%. Other causes of slow growth include: microbial contamination, too frequent subculture, too low density seeding at subculture, senescence of cell line, change in medium composition, and incubator inadequacy in regulating temperature, humidity or CO₂

How should a newly received fibroblast cell culture be handled?**Procedure:**

1. Observe cell sheet for confluency, morphology of cells and signs of contamination. Wipe culture flasks with a disinfecting solution and place in a 37°C incubator overnight with the cell sheet down. Do not remove medium (contains only 5% FBS to slow growth during transport).
2. The next day, examine the flask(s) as indicated above, and depending on the culture's confluency, either feed the flask by withdrawing the shipping medium and covering the cells with ~5 ml growth medium, or sub-culture the cells according to the procedure outlined below.
3. When sub-culturing a newly received fibroblast culture, the correct passage number must be determined. If the passage number is noted on the submission sheet or flask, the sub-cultured flasks should receive the next consecutive passage number.

How is a fibroblast cell line sub-cultured?**Supplies**

- 0.53 mM EDTA in HBSS
- 0.04% trypsin/0.53 mM EDTA in HBSS
- Fibroblast Growth medium

Procedure:

1. Remove medium by aspiration.
2. Refer to Table 1 for the proper volume of reagents to use.
3. Add the appropriate volume of EDTA solution to the flask without dislodging the cell sheet and lay the flask cell side down.

4. Watch cells closely through an inverted microscope for up to 10 minutes. If the cells begin to round or lift off the flask, remove the EDTA solution immediately.
5. Replace the EDTA solution with the EDTA/trypsin solution.
6. Incubate the flasks at 37°C for 4-7 minutes. The cells will round up and detach from the surface of the flask.
7. Tighten cap and lightly tap the side of the flask to lift the remaining cells from the flask.
8. Examine the flasks microscopically to make sure the cells have all detached. If the cells do not become detached after seven minutes, incubate an additional 1 to 2 minutes.
9. Wash the back of the flask with growth medium (Stop Medium) to inactivate the trypsin, and gently mix the cells and medium.
10. Remove an aliquot for a cell count, and count the cells.
11. Seed the flasks according to Table 2.
12. Place flasks in an incubator set to the appropriate parameters for the individual cell line (typically 37° C, 5% CO₂), loosen caps if not vented.
13. Check the cultures after a few hours for cell attachment and pH. The time between sub-cultures will depend on the cell line. The majority of mammalian cell lines require sub-culturing every 5-7 days. If the duration is longer than 5 days, change the culture medium every 3-4 days.

Table 1: Reagent Volumes

Flask Size	EDTA (ml)	Trypsin/EDT A Solution (ml)	Stop Medium (ml)
T12.5	2	2	≥ 2
T25	3	3	≥ 3
T75	5	5	≥ 5
T175	10	10	≥ 10

Note: Stop medium can be any serum-containing growth medium, but the same growth medium required by the cell line is what is typically used.

Table 2: Flask Volume and Seeding Density

Flask Size	Final Volume	Seeding Density (Viable cells per flask)
T12.5	3-5 ml	1.0 x 10 ⁵ – 2.5 x 10 ⁵

T25	4-10 ml	$2.0 \times 10^5 - 6.0 \times 10^5$
T75	15-25 ml	$8.5 \times 10^5 - 1.3 \times 10^6$
T175	35-50 ml	$1.8 \times 10^6 - 2.4 \times 10^6$

Note: The chart above is to be used as a guideline only. Basic seeding rule is $1.0-1.4 \times 10^4$ cells/cm².

How are fibroblast cultures frozen for cryogenic storage?

Supplies

- 0.53mM EDTA in HBSS
- 0.04% trypsin/0.53 mM EDTA in HBSS
- Fibroblast Growth medium
- Fibroblast Freeze medium (growth medium with 10% glycerol or 5% DMSO)

Procedure

1. If cells are to be frozen in 10% glycerol, complete freeze medium may be kept at room temperature until used. Freeze medium prepared with DMSO should be kept refrigerated until used.
2. Examine each flask that is to be pooled for the freeze (freeze pool) microscopically for contamination and any unusual growth pattern. One flask should be maintained as a “backup” flask until the viability of the freeze can be checked.
3. Follow sub-culturing steps 1-9 above for each flask.
4. Transfer cell suspension from all flasks and pool cells in a centrifuge bottle sitting on ice.
5. Remove an aliquot of the freeze pool for count, count the cells and calculate the total viable cells in the freeze pool.
6. Centrifuge the freeze pool at 60-100 x g for 10 minutes at 8-10°C.
7. Remove the supernatant and re-suspend the cell pellet using gentle trituration in freeze medium at a final concentration of at least 5×10^5 viable cells per ml.
8. Distribute 1 ml aliquots of the cell suspension into glass ampoules or plastic cryovials.
9. Seal glass ampoules using an oxygen-propane flame. Check each glass ampoule for pinholes or glass bubbles formed during sealing by immersion in a methylene blue/ethanol solution at 4°C.
10. Freeze the ampoules or cryovials at a rate of -1°C per minute to -80°C (either in microprocessor controlled freezer or passively in an isopropanol bath placed in a -80°C freezer overnight).
11. Store frozen cell stocks in liquid nitrogen. Submerge glass ampoules in liquid; store plastic cryovials in the vapor phase.

How should fibroblast cell cultures be recovered from cryogenic storage?

Procedure

1. Prepare appropriate growth medium.
2. Remove one ampoule or cryovial from frozen storage and place immediately in a 37°C water bath and agitate vigorously.
3. Once completely thawed, disinfect ampoule or cryovial with a 70% alcohol sponge, or equivalent disinfectant. Score the neck of a glass ampoule with a file and open utilizing an ampoule opener.

4. Remove the contents of the ampoule or cryovial using a sterile transfer pipette and place in a T25 tissue culture flask containing 5 ml of the appropriate fresh growth medium.
5. If a cell count is required, mix the contents of the flask gently with a 1 ml pipette and remove 0.2 ml for a 1:5 diluted cell count. Place the flask in the appropriate incubator lying cell surface down. Gently swirl the flask to distribute the cell suspension evenly over the flask surface. Adjust the cap to allow appropriate gas exchange (depending on buffering system of the medium).
6. Fibroblast cultures should be re-fed with fresh medium the day after recovery.
7. Some cell lines recover better if all traces of cryoprotectant are removed by washing and centrifugation. Transfer the contents of ampoule or cryovial to a 15-ml centrifuge tube with 3-5 ml of growth medium.
8. Centrifuge for 5 min at 60-100xg and 8-10°C.
9. Remove supernatant, re-suspend pellet, and transfer to a T25 flask with a final volume of ~5 ml.
10. Culture as needed as outlined above for sub-culturing fibroblasts. If cells fail to proliferate after 1-2 weeks, expand the backup flask for a second freeze.