# HFF feeder cell protocol

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## Human fetal fibroblast (HFF) Mitomycin-C treated, Research grade

| | |
|---|---|
| Cell Source | Human fetal dermal fibroblast |
| Treatment | Mitomycin-C treated |
| Cryopreservation Medium | 10% DMSO/40% FBS in DMEM |
| Growth Medium | DMEM with 10% FBS |
| Storage | Vapor phase of liquid nitrogen |
| Shipping | Dry ice |

## Intended Use:

Mitotically inactivated fibroblast is used as a support layer for cell culture. These cells should be plated 24 hours prior to use and cultured up to 7 days.

## Caution:

These cells are only for research use only and not intended for human or animal diagnostic or therapeutic use.

## Procedures:

1. Put 9 mL of growth medium in a centrifugation tube.
2. Place the frozen vial into 37℃ water bath immediately and it takes about 2 minutes to thaw completely.
3. Transfer the contents of the vial to the centrifugation tube to make a 1:10 dilution with growth medium.
4. Centrifuge the tube at RT (20~25)℃, 230g for 7 minutes to pellet the cells.
5. Resuspend the pellet in growth medium and seed the cells in tissue culture dish at appropriate density*.
6. Place the tissue culture dish with feeder cells in cell culture incubator (37℃, 5%CO2) for 24 hours.
7. The feeder cells are now ready to use.

*The suitable seeding density should be determined by users.

## Plating density:

| Type of Vessel | CBI010120<br>1.5 - 2M x $10^6$ cell/vial<br>Number of Plate | CBI010140<br>3.5 - 4M x $10^6$ cell/vial<br>Number of Plate |
|---|---|---|
| 6-well plates | 1 – 2 | 2 – 3 |
| 12-well plates | 1 – 2 | 3 – 4 |
| 10 cm dish | 1 – 2 | 2 – 3 |
| T75 flask | 1 | 2 |

*Suggesting density: 2 – 3 x $10^4$ cells/$cm^2$

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