IVISbrite LNCaP red F-luc tumor cell culture guidelines

IVISbrite™ LNCaP Red F-luc tumor cells (BW125055) is a mixture of adherent and semi-adherent cells that requires special culture techniques. Please follow the instructions below carefully.

In all instances where HyClone™ FBS is mentioned, we strongly recommend HyClone™ Characterized Fetal Bovine Serum (U.S. origin, no processing. Cytiva™ Cat. No. SH30071). Do not use heat-inactivated or irradiated FBS as it may inhibit cell growth.

Thawing a frozen cell vial

Note: All manipulations of cells must be performed under aseptic conditions.

1. Thaw 1 vial in 37 °C water bath with brisk agitation. Vial should be thawed quickly within 2 minutes.
2. Immediately upon thaw, transfer contents of vial into T-25 tissue-culture treated flask containing 8 mL RPMI-1640 medium + 10% HyClone™ FBS (without any antibiotics). The medium should be warmed and acclimated in flask in tissue culture incubator 37 °C/5%CO_2 for 15 minutes prior to cell addition.
3. Gently swirl flask once to mix cells then remove 1 mL of cell suspension in order to obtain cell count and viability. Do not pipette cells strongly. Incubate flask at 37 °C/5%CO_2 for at least 24-48 hours before disturbing flask.

4. Observe flask under microscope at 48 hours. If flask is less than 80% confluent, gently remove media and add fresh warmed media. This cell line tends to be a mixture of adherent and semi-adherent cells.
5. If there is a large amount of floating or semi-adherent clumps of cells, transfer the supernatant including the floating cells into a new flask of the same size and wait 48 hours to see if these cells will adhere. Continue to incubate the flasks until they reach 80% confluence before attempting to passage. These cells are very slow growing especially upon recovering from thaw and it is not unusual for passage intervals to be between 5-8 days.
6. During the incubation period, it is important to change the medium every 48 hours or so, either transferring floating cells to another flask or discarding them.

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Passaging cell lines

Recommended medium: RPMI-1640 medium + 10% HyClone™ FBS + Puromycin at 2 ug/mL.

For *in vivo* use we recommend less than 10 *in vitro* passages from original vial. However, split cells at least one time before injecting *in vivo*.

1. To passage cells upon reaching 80% confluency, gently aspirate and discard supernatant.
2. Carefully add appropriate amount of 0.25% Trypsin-EDTA to flask. Tilt the flask gently to rinse the cells with the Trypsin solution without dislodging any cells. Discard this rinse.
3. Carefully add another quantity of 0.25% Trypsin-EDTA to the flask and place in 37 °C/5% CO_2 incubator for 5 minutes. Do not swirl or agitate flask at any time since these cells will tend to clump.
4. Observe under microscope to ensure that cells have detached from surface of flask. It is normal for cells to detach as strings of cells and/or small clumps. If necessary, incubate for an additional 5 minutes.
5. Add a 4x quantity of medium into flask, tilting flask to rinse surface, and transfer cell suspension into a sterile conical tube. Be careful to avoid excess pipetting action as this may damage cells – DO NOT pipette cells vigorously at this point in an attempt to disperse clumps.
6. Spin tube in centrifuge at 1000 rpm for 10 min.
7. Decant supernatant, then rapidly tap bottom of conical tube against hard surface to break up cell pellet (in remaining drop or two of media left in tube). Tap until pellet no longer contains clumps – this will not damage cells.
8. Add fresh medium to conical tube to resuspend pellet – take care to only pipette minimally.
9. Perform cell count on sample of resuspended cells. Add appropriate quantity of cell suspension to new flasks containing fresh medium. The approximate split ratio is 1:3 - 1:5.
10. As before, allow cells to incubate for a minimum of 48 hours.
11. Change medium and continue to incubate another 48-96 hours until 80% confluency is reached.
12. Continue to change medium every 48 hours during interval between passages.
13. Perform next passage, being careful to avoid swirling or agitating flasks during trypsinization and being careful to avoid any excess pipetting action at any time. If a decrease in viability is noted or large clumps form during the passaging process, try to minimize agitation of flasks and pipette only at a minimum. Once established in flask, these cells tend to grow as single, elongated adhered cells with clusters of small adhered or semi-adhered clumps of cells in between.

Creating cell stocks

1. When cells have reached 80% confluence, harvest as above for passage except in medium without antibiotics. Centrifuge cell suspension at 1000 rpm for 10 minutes. Resuspend cells at appropriate concentration in ice-cold freezing medium (5% DMSO/95% FBS without antibiotics). Aliquot cell suspension into cryogenic storage vials, then place the cryovials in an insulated freezer box at -80 °C. After 24 hours, transfer frozen vials to liquid nitrogen storage.
2. After a day or two, retrieve one vial for thawing and culture to ensure that the batch of frozen cells retained proper viability.

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