

SOP: Propagation of Kc167

Date modified: 2025/11/4 Modified by: YangYang

Name: Kc167

Source: cloned from Kc

Species: *Drosophila melanogaster*

Sex of cell: female

Culture Properties: semi-suspension.

Medium for Kc167

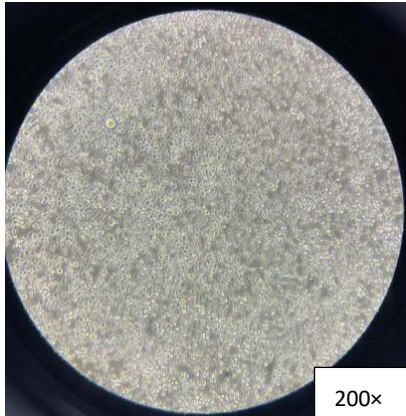
1. Growth medium: 90% Gibco™ Schneider's Drosophila Medium (Gibco, Cat. no. 21720024) + 10% FBS (ExCell Bio, Cat. no. FSP500).
2. Freezing medium: 45% conditioned complete Schneider's Drosophila Medium containing 10% FBS, 45% fresh complete Schneider's Drosophila Medium containing 10% FBS, and 10% DMSO.

Culture conditions

1. Stilling culture: for example-- in T-25 cm² flask with 5 ml medium upright position.
2. 27°C under 5% CO₂ or without CO₂.

Initiate Kc167 cell culture from frozen stock and passage cells

1. **Initiate:** Pre-warm growth medium at 27°C at least 1 h before use.
2. Thaw the cells (1 ml, 1*10⁷ cells/tube) in 27°C water bath (~2 min). Decontaminate the outside of the vial with 75% ethanol. Transfer the cells immediately into T-25 cm² flask containing 4 ml pre-warmed fresh growth medium.
3. Place the flask in a 27°C incubator with 5% CO₂ or without CO₂ for 16-24 h.
4. Transfer the 5 ml of cell suspension into a 15-ml tube, and immediately add 4 ml of pre-warmed fresh growth medium into the flask.
5. Centrifuge the 15-ml tube at 800 rpm for 5 minutes. Aseptically decant the medium containing DMSO and resuspend the cell pellet in 1 ml of pre-warmed fresh growth medium and transfer to the flask.
6. Check cells morphology under microscope, and check cell density and viability via CounterStar every other day. Cells will start to clump at a density of ~5 × 10⁶ cells/ml in serum-containing medium. This does not seem to affect growth. Clumps can be broken up during passage.
7. **Passage:** After recovery, they will need to be passaged every 3-4 days. Once they reach approximately a density of 1-2*10⁷ cells/ml, they should be passaged. Transfer the whole cells to a new flask, add pre-warmed fresh growth medium to a cell density of 2-5*10⁶ cells/ml, and place the flask in a 27°C incubator with 5% CO₂ or without CO₂ for stilling culture.



Freeze Kc167 cells

IMPORTANT! Optimal recovery of Kc167 cells requires growth factors in the medium. Be sure to use conditioned medium in the Freezing Medium.

1. Before starting, label ~10 cryovials and prepare 5.5 ml of partial Freezing medium in a 15-ml tube: 4.5 ml of growth medium + 1 ml of DMSO.
2. When cells are between 1.0×10^7 – 1.5×10^7 cells/ml and viability is 97–99% in flask, transfer 10 ml of cell culture ($\sim 10 \times 10^7$ cells) into a 15 ml tube.
3. Spin down the 15-ml tube at 800 rpm for 5 min at RT.
4. Transfer 4.5 ml of the supernatant (conditioned medium) to the partial Freezing medium tube to prepare Freezing medium and discard remaining 5.5 ml supernatant.
5. Re-suspend the cells with 10 ml of Freezing medium ($\sim 1 \times 10^7$ cells/ml).
6. Aliquot 1 ml of the cell suspension per vial.
7. Freeze cells in a control rate freezer to -80°C .
8. Transfer container to -80°C and hold for 24 hours to allow for a slow freezing process.
9. Transfer vials to liquid nitrogen for long-term storage.

References

<https://dgrc.bio.indiana.edu/cells/modencode/Protocol-Kc167>