SOP: Propagation of *Drosophila* Schneider 2 (S2)

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Name: *Drosophila* Schneider 2 (S2)

Tissue Source: late stage (20–24 hours old) *Drosophila melanogaster* embryos

Species: *Drosophila melanogaster*

Sex of cell: male

Culture Properties: Loose, semi-adherent monolayer in tissue culture flasks and in suspension in spinners and shake flasks.

Information: The S2 cell line was derived from a primary culture of late stage (20–24 hours old) *Drosophila melanogaster* embryos. Many features of the S2 cell line suggest that it is derived from a macrophage-like lineage. S2 cells grow at 26°C–28°C without CO_2 as a loose, semi-adherent monolayer in tissue culture flasks and in suspension in spinners and shake flasks.

Medium for S2

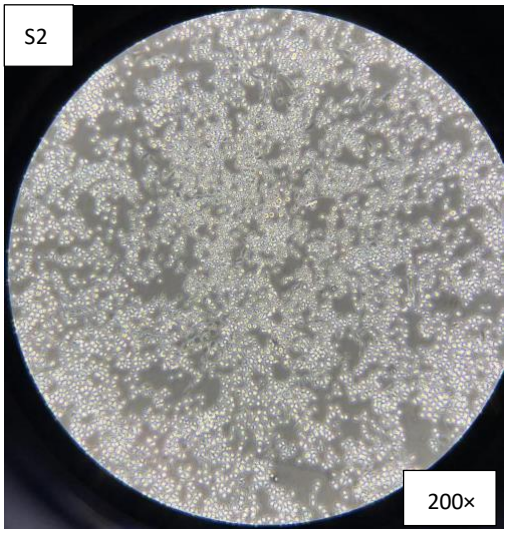
1. Growth medium: 90% Gibco™ Schneider's Drosophila Medium (Gibco, Cat. no. 21720024) + 10% FBS (ExCell Bio, Cat. no. FSP500).
2. Freezing medium: 45% conditioned complete Schneider's Drosophila Medium containing 10% FBS, 45% fresh complete Schneider's Drosophila Medium containing 10% FBS, and 10% DMSO.

Culture conditions

1. Stilling culture: for example--in T-25 cm^2 flask with 5 ml medium upright position.
2. Suspending culture: in Erlenmeyer flask with 20-50 ml medium with shake (80-90 rpm).
3. 27°C under 5% CO_2 or without CO_2.

Initiate S2 cell culture from frozen stock and passage cells

1. **Initiate:**Pre-warm growth medium at 27℃ at least 1 h before use.
2. Thaw the cells (1 ml, 1*10^7 cells/tube) in 27℃ water bath (~2 min). Decontaminate the outside of the vial with 75% ethanol. Transfer the cells immediately into T-25 cm^2 flask containing 4 ml pre-warmed fresh growth medium.
3. Place the flask in a 27℃ incubator with 5% CO_2 or without CO_2 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 at RT. 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 × 10^6 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*10^7 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*10^6 cells/ml, and place the flask in a 27℃ incubator with 5% CO_2 or without CO_2 for stilling culture or place the flask in a 27℃ incubator without CO_2 for suspending culture.



Freeze S2 cells

IMPORTANT! Optimal recovery of S2 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 (~10*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 (~1*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

Gibco *Drosophila Schneider* 2 (S2) Cells User Guide https://www.thermofisher.cn/order/catalog/product/cn/zh/R69007