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Drosophila Schneider 2 (S2) Cells

USER GUIDE

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ThermoFisher SCIENTIFIC

NOTE TO WRITER: ADD CONKEYREF SOURCE TO PUBLICATION

The information in this guide is subject to change without notice.

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Revision	Date	Description
D.0	09 December 2019	Updating details in Culture S2 Cells topics.
C.0	27April 2018	Update density in Passage S2 cells
B.0	14 March 2017	Rebrand and update legal boilerplates
A.0	31 March 2015	Baseline for this revision

Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

Contents

Product information

Introduction

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.

Contents and storage

Table 1 *Drosophila* Schneider 2 (S2) Cells (Cat. No. R69007)

Contents	Amount	Storage [1]
Drosophila Schneider 2 (S2) Cells, 1 × 10^7 cell/mL[2]	1 mL	Liquid nitrogen

[1] Cells are shipped on dry ice.

[2] Cells are supplied in Freezing Medium (45% conditioned complete Schneider's *Drosophila* Medium containing 10% heat-inactivated fetal bovine serum [FBS], 45% fresh complete Schneider's *Drosophila* Medium containing 10% heat-inactivated fetal bovine serum, and 10% DMSO).

Required materials not supplied

All materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Schneider's *Drosophila* Medium	21720001
Complete Schneider's *Drosophila* Medium[1]	—
Calcium Phosphate Transfection Kit	K278001
Hygromycin-B	10687010
Blasticidin S HCl	R21001
DES™–Inducible/Secreted Kit with *pCoHygro*	K413001
DES™–Inducible/Secreted Kit with *pCoBlast*	K513001
DES™– Inducible Kit with *pCoHygro*	K412001
DES™– Inducible Kit with *pCoBlast*	K512001
DES™– Constitutive Kit with *pCoHyro*	K411001

Item	Source
DES™ – Constitutive Kit with *pCoBlast*	K511001
15-mL sterile, conical tubes	MLS
5-, 10-, and 25-mL sterile pipettes	MLS
Cryovials	MLS
Hemocytometer and Trypan blue	MLS
25-cm^2 flasks, 75-cm^2 flasks, and 35-mm plates[2]	MLS

[1] Contains 10% heat-inactivated fetal bovine serum (FBS). Pluronic F-68 should also be added at 0.1%, if culturing suspension cells.

[2] Other plates and flasks may be used.

Culture S2 cells

Procedural guidelines

All solutions and equipment that come in contact with the cells must be sterile.

- Always use proper sterile technique in a laminar flow hood.
- All incubations are performed in a 26°C–28°C incubator and do not require CO^2.

  Note: Ideal growth temperatures for insect cells are 26°C–28°C. Cooler temperatures (<26°C) will provide slower cell growth. Warmer temperatures (> 28°C) will initially provide slow growth followed by issues with cell viability.
- The complete medium for S2 cells is Schneider's *Drosophila* Medium containing 10% heat-inactivated FBS and 0.1% Pluronic™ F-68. This medium is used for transient expression and stable selection.

  Note: Pluronic™ F-68 is required for suspension culture but not required for adherent cultures.
- Optional: Use Penicillin-Streptomycin at a final concentration of 50 units penicillin G and 50 µg streptomycin sulfate per milliliter of medium.
- Before starting experiments, be sure to have established frozen S2 cell stocks.
- Count cells before seeding for transfection or freezing cells for stocks. Check for viability using trypan blue. S2 cell viability in culture should be 95–99%.
- Always use new flasks or plates when passing cells for general maintenance. During transfection and selection keep cells in the same culture vessel.
- For general maintenance of cells, refer to adherent and suspension culture sections of this user guide.
- Cell doubling time is about 24 hours when cells are routinely passaged in log to mid-log phase of growth.

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.
- Prior to starting experiments, ensure you have established cells and have frozen stocks on hand. Upon receipt, grow and freeze multiple vials of cells to ensure that you have an adequate supply of early-passage cells.

Initiate cell culture from frozen stock

The vial of S2 cells supplied contains ~1 × 10^7 cells. Upon thawing, cells should have a viability of 60–70%. Once the culture is established, cell viability should be >95%.

1. Remove the vial of cells from liquid nitrogen and thaw quickly at 30°C.

   Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol.

2. Triturate and transfer the entire contents of the cryovial into a sterile, conical tube containing 4 mL of room temperature, complete Schneider's *Drosophila* Medium.

3. Centrifuge at 125-200 × *g* for 5–10 minutes. Aseptically decant the medium containing DMSO and gently resuspend the cell pellet in 5 mL of fresh complete Schneider's *Drosophila* Medium.

4. Transfer the 5 mL of cell suspension into a 125-mL shake flask containing 20 mL of fresh complete Schneider's *Drosophila* Medium containing 0.1% Pluronic™ F-68. Incubate cells in a 28°C non-humidified, ambient air-regulated incubator or warm room. If using a non-vented cap flask, loosen the caps of flasks to allow oxygenation/aeration.

   Note: If cells are to be cultured in semi-adherent conditions, we recommended transferring the cell suspension into a T-175 flask containing 35 mL of fresh complete Schneider's *Drosophila* Medium.

5. Incubate at 28°C until cells reach a density of 0.6 × 10^7–2.0 × 10^7 cells/mL (or between 5 × 10^4 and 4 × 10^5 cells/cm^2 if culturing in a semi-adherent format.

   This may take 3–4 days.

Passage S2 cells

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.

1. S2 cells should be subcultured to a final density of 2 × 10^6–4 × 10^6 cells/mL (typical subcultivation ratio of 1:5 to 1:10 or greater). Do not split cells below a density of 0.5 × 10^6 cells/mL. For example, 2 mL of cells from a 75-cm^2 flask at a density of 2 × 10^7 cells/mL should be placed into a new 75-cm^2 flask containing 10 mL of fresh complete Schneider's *Drosophila* Medium. Similarly, for a suspension culture, 4 mL of cells from a 125-mL shake flask at a density of 2 × 10^7 cells/mL should be placed into a new 125-mL shake flask containing 21 mL of fresh complete Schneider's *Drosophila* Medium containing 0.1% Pluronic™ F-68.

2. For semi-adherent cultures, when removing cells from the flask, tap the flask several times to dislodge cells that may be attached to the surface of the flask.

   Use a 5-mL pipette to wash down the surface of the flask with the conditioned medium to remove the remaining adherent S2 cells.

3. Once the cells have detached, briefly pipette the solution up and down to break up clumps of cells.

4. Split cells at a 1:10 dilution into new culture vessels, then add complete Schneider's *Drosophila* Medium and incubate at 28°C incubator until the density reaches 0.6×10^7–2.0×10^7 cells/mL.

5. Repeat Steps 1–4 as necessary to expand cells for transfection or expression

Freeze S2 cells

Before starting, label ~15 cryovials and place on wet ice.

Freezing Medium is 45% conditioned complete Schneider's *Drosophila* Medium containing 10% heat-inactivated FBS, 45% fresh complete Schneider's *Drosophila* Medium containing 10% heat-inactivated FBS, and 10% DMSO. Be sure to reserve medium after centrifuging cells.

IMPORTANT! Optimal recovery of S2 cells requires growth factors in the medium. Be sure to use conditioned medium in the Freezing Medium. In addition, FBS that has not been heat-inactivated will inhibit growth of S2 cells.

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1. When cells are between 1×10^7–2×10^7 cells/mL in a 75-cm^2 flask, remove the cells from the flask.

   There should be 12 mL of cell suspension.

2. Count a sample of cells in a hemacytometer to determine actual cells/mL and the viability (95–99%).

3. Pellet the cells by centrifuging at 125-200 × *g* for 5–10 minutes in a table top centrifuge at 4°C. Reserve the conditioned medium.

4. Resuspend the cells in 10 mL PBS and pellet at 125-200 × *g* for 5–10 minutes.

5. Prepare Freezing Medium (see recipe this section).

6. Resuspend the cells at a density of 1.1×10^7 cells/mL in Freezing Medium.

7. Aliquot 1 mL of the cell suspension per vial.

8. Freeze cells in a control rate freezer to –80°C.

9. Transfer container to –80°C and hold for 24 hours to allow for a slow freezing process.

10. Transfer vials to vapor-phase liquid nitrogen for long-term storage.

Transfect S2 cells

Introduction

Drosophila Schneider 2 (S2) cells can be transfected with the recombinant expression vector alone for transient expression studies or in combination with a selection vector (e.g., pCoHygro or pCoBlast) to generate stable cell lines. We recommend that you test for expression of your protein by transient transfection before undertaking selection of stable cell lines. Once you have demonstrated that your protein is expressed in S2 cells, you can create stable transfectants for long-term storage, increased expression of the desired protein, and large-scale production of the desired protein. *Drosophila* stable cell lines generally contain multicopy inserts that form arrays of more than 500–1000 copies in a head to tail fashion. The number of inserted gene copies can be manipulated by varying the ratio of expression and selection plasmids. We recommend using a 19:1 (w/w) ratio of expression vector to selection vector. You may vary the ratio to optimize expression of your particular gene. Transfection using calcium phosphate is recommended, but some lipid-based transfection reagents are also suitable. Go to **thermofisher.com**

Procedural guidelines

- The first time you perform a transient transfection you may wish to perform a time course to ensure that you detect expression of your protein. We suggest assaying for expression at 2, 3, 4, and 5 days posttransfection.
- **Vector selection**: The DES™ kits are available with a choice of pCoHygro or pCoBlast selection vectors (see Required Materials). The pCoHygro and pCoBlast selection vectors express the hygromycin or blasticidin resistance genes, respectively from the copia promoter. See the DES™ manual for more information. Other selection vectors can be used.
- **Antibiotic selection:** To select for S2 cells that have been stably cotransfected with pCoHygro and a DES™ expression vector, we generally use 300 µg/mL hygromycin-B. For S2 cells stably cotransfected with pCoBlast and a DES™ expression vector, we use 25 µg/mL blasticidin. Selection with hygromycin generally takes 3–4 weeks, while selection with blasticidin generally takes only 2 weeks. Cell death may be verified by trypan blue staining. If you are using another selection vector, use the recommended concentration of selection agent or perform a kill curve.
  - Prepare complete Schneider's Drosophila Medium supplemented with varying concentrations of selection agent.
  - Test varying concentrations of selection agent on the S2 cell line to determine the concentration that kills your cells (kill curve).

Before you begin

1. The first time you perform a transient transfection you may wish to perform a time course to ensure that you detect expression of your protein. We suggest assaying for expression at 2, 3, 4, and 5 days posttransfection.

2. Have the following reagents and equipment ready before starting:
   - S2 cells growing in culture (3×10^6 S2 cells/well in a 35-mm plate per transfection)
   - 35-mm plates (other flasks or plates can be used)
   - Complete Schneider's Drosophila Medium
   - Recombinant DNA (19 µg per transfection. May be varied for optimum expression.)
   - pCoHygro, pCoBlast, or other selection vector (1 µg per transfection)
   - Sterile microcentrifuge tubes (1.5-mL)
   - Lysis Buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P-40, pH 7.8)
   - Calcium Phosphate Transfection Kit (included in the DES™ Kit or available separately, Cat. no. K2780-01)

Calcium phosphate transfection

Instructions are for one transfection per 35-mm plate. You may want to include additional plates for time points after transfection. We recommend that you include a negative control (empty vector) and a positive control (included with the DES™ kit of choice). We recommend that you also test for expression of your protein before selecting for a stable population.

Day 1: Preparation

1. Prepare cultured cells for transfection by seeding 3×10^6 S2 cells (1×10^6 cells/mL) in a 35-mm plate in 3 mL complete Schneider's *Drosophila* Medium.

2. Grow 6–16 hours at 28°C until cells reach a density of 2×10^6–4×10^6 cells/mL.

Day 2: Transient transfection

Prepare the following transfection mix (per 35-mm plate). Include the selection vector only if generating stable cell lines.

1. In a microcentrifuge tube mix together the following components to make **Solution A**:

Component	Amount
2 M $CaCl_2$	36 µL
Recombinant DNA (19 µg)	X µL
Selection vector (1 µg) (optional)	Y µL
Tissue culture sterile water	Bring to final volume of 300 µL

2. In a second microcentrifuge tube, add 300 µL 2X HEPES-Buffered Saline (50 mM HEPES, 1.5 mM Na_2HPO_4, 280 mM NaCl, pH 7.1). This is **Solution B**.

3. Slowly add **Solution A** dropwise to **Solution B** with continuous mixing (you may vortex or bubble air through the solution). Continue adding and mixing until **Solution A** is depleted. This is a slow process (1–2 minutes). Continuous mixing ensures production of the fine precipitate necessary for efficient transfection.
4. Incubate the resulting solution at room temperature for 30–40 minutes.
   After ~30 minutes a fine precipitate should form.
5. Mix the solution and add dropwise to the cells.
   Swirl to mix in each drop.
6. Incubate 16–24 hours at 28°C.
   Note: You may wish to investigate whether extending the incubation time improves transfection efficiency.

Calcium phosphate transfection (transient)

Day 3: Post-transfection (transient expression)

1. Remove calcium phosphate solution and wash the cells twice with complete medium.
2. Add fresh, complete Schneider's *Drosophila* Medium and replate into the same vessel.
   Continue to incubate at 28°C.
3. If you are using an inducible expression vector (e.g., pMT/V5-His or pMT/BiP/V5-His), induce expression when the cells either reach log phase (2×10^6–4×10^6 cells/mL) or 1 to 4 days after transfection.
4. Add copper sulfate to the medium to a final concentration of 500 µM.
   For example, to induce a 3 mL culture, add 15 µL of a 100 mM $CuSO_4$ stock. Induce for 24 hours before assaying protein.

Day 4+: Harvest cells (transient expression)

Harvest the cells 2, 3, 4, and 5 days post-transfection and assay for expression of your gene.
There is no need to add fresh medium or additional inducer.

Calcium phosphate transfection (stable)

Day 3: Post-transfection (stable expression)

1. Remove the calcium phosphate solution and wash the cells twice with complete medium.
2. Add fresh complete Schneider's *Drosophila* Medium (no selection agent) and replate into the same well or plate.
   Do not split cells.
3. Incubate at 28°C for 2 days.

Day 5: Selection (stable transfection)

1. Centrifuge cells and resuspend in complete Schneider's *Drosophila* Medium containing the appropriate selection agent.
2. Replace selective medium every 4–5 days until resistant cells start growing out (generally varies between 2–4 weeks depending on the selection agent you are using).

   Always replate into old plates.

+2–3 weeks: Expansion (stable transfection)

1. Centrifuge cells and resuspend in complete Schneider's *Drosophila* Medium containing the appropriate selection agent.
2. Passage cells at a 1:2 dilution when they reach a density of 6×10^6–20×10^6 cells/mL.

   This is to remove dead cells.

   Note: You may want to plate resistant cells into smaller plates or wells to promote cell growth before expanding them for large-scale expression or preparing frozen stocks.
3. Expand resistant cells into 6-well plates to test for expression .

   Always use complete Schneider's *Drosophila* Medium containing the appropriate concentration of selection agent when maintaining stable S2 cell lines.

Test for expression

Use the cells from one 35-mm plate for each expression experiment. Cells may be transiently or stably transfected.

1. Prepare an SDS-PAGE gel that will resolve your expected recombinant protein.
2. Transfer cells to a sterile, 1.5-mL microcentrifuge tube.

   If your protein is secreted, be sure to save and assay the medium.
3. Pellet cells at 1000 × g for 2–3 minutes, then transfer the supernatant (medium) to a new tube and resuspend the cells in 1 mL PBS.
4. Pellet cells and resuspend in 50 µL Lysis Buffer.
5. Incubate the cell suspension at 37°C for 10 minutes.

   Note: You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
6. Vortex and pellet nuclei and cell debris, then transfer the supernatant to a new tube.
7. Assay the lysate for the protein concentration.
8. Mix the lysate or the medium with SDS-PAGE sample buffer.

9. Load approximately 3–30 µg protein per lane.

   Amount loaded depends on the amount of your protein produced. Load varying amounts of lysates or medium.

10. Electrophorese your samples, blot, and probe with antibody.

11. Visualize proteins using your desired method.

    We recommend using chemiluminescence or alkaline phosphatase for detection.

Troubleshooting

Observation	Possible cause	Recommended action
Cells growing too slowly (or not at all)	Cells were split back too far.	Do not plate cells at less than 0.5×10^6 cells/mL. Cells will eventually grow back up if they weren't split back too far.
		Replate new cells.
	Conditioned medium was not used.	Bring conditioned medium along during passage.
Low transfection efficiency	Purification and transfection methods	Use clean, pure DNA isolated by CsCl gradient ultracentrifugation or the S.N.A.P.™ MidiPrep Kit (Cat. no. K1910-01).
		Make sure the calcium phosphate precipitate is fine enough. Be sure to thoroughly and continuously mix **Solution B** while you are adding **Solution A**.
		Try a different method of transfection. Go to **thermofisher.com** (Lipid-mediated transfection).
Low or no protein expression	Gene or protein not in frame with signal sequence.	If using secretion vector, clone gene in-frame with signal sequence. If protein is not in frame with the signal sequence, it will not be expressed or secreted.
	No Kozak sequence for proper initiation of transcription.	Include Kozak sequence for proper initiation of transcription. Translation will be efficient and protein expressed efficiently.
	Gene product is toxic to S2 cells.	Use a vector (e.g., pMT/V5-His or pMT/BiP/V5-His) for inducible expression.

Additional guidelines

Inducers: Other researchers have used 10 µM $CdCl_2$ to induce the metallothionein promoter. While cadmium is an effective inducer, note that cadmium will also induce a heat shock response in *Drosophila*. In addition, higher concentrations of copper sulfate (600 µM to 1 mM) have been used to induce some protein.

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.