USER GUIDE

invitrogen
by Thermo Fisher Scientific

Drosophila Schneider 2 (S2) Cells

Catalog Number R690-07

Document Part Number 28-0172
Publication Number MAN0000656
Revision A.0

ThermoFisher
SCIENTIFIC

Information in this document is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

LIMITED USE LABEL LICENSE No. 561: Internal Research and Development Use of Mammalian and Insect Cell Lines

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product and progeny, to perform internal research and development for the sole benefit of the purchaser. The purchase of this product does not grant the purchaser any additional rights, including, without limitation: (a) the right to use the product or its components in commercial applications of any kind, including bioproduction (e.g. use of the product to manufacture therapeutic agents or diagnostic test components, such as proteins, antibodies, viral particles or virus-like particles), quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration; (b) the right to use the product or its components as a therapeutic agent or diagnostic test component; (c) the right to use the product or its components to produce material for use in human clinical trials; or (d) the right to transfer or resell the product or its components in any form, progeny or derivative. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008.

Table of contents

Product information

Shipping and storage

Cells are shipped on dry ice. Upon receipt, store the cells in liquid nitrogen.

Kit contents

One vial of Schneider 2 (S2) cells is supplied (1 mL per vial, 1×10^7 cells/mL) 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).

Products available separately

The following DES™ products are available separately from Thermo Fisher Scientific.

Product	Amount	Catalog no.
Schneider's *Drosophila* Medium	500 mL	21720-024
Calcium Phosphate Transfection Kit	75 reactions	K2780-01
Hygromycin-B	1 gram	R220-05
Blasticidin S HCl	50 mg	R210-01
DES™- Inducible/Secreted Kit		
with pCoHygro	1 kit	K4130-01
with pCoBlast	1 kit	K5130-01
DES™- Inducible Kit		
with pCoHygro	1 kit	K4120-01
with pCoBlast	1 kit	K5120-01
DES™- Constitutive Kit		
with pCoHygro	1 kit	K4110-01
with pCoBlast	1 kit	K5110-01

Methods

Culture S2 cells

Introduction

The S2 cell line was derived from a primary culture of late stage (20–24 hours old) *Drosophila melanogaster* embryos (Schneider, 1972). 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.

General cell handling

General guidelines are provided to help you grow S2 cells.

- 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. Schneider's *Drosophila* Medium is available separately (Cat. no. 21720-024).

  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.

Before starting

Be sure to have the following solutions and supplies available:

- 15-mL sterile, conical tubes
- 5-, 10-, and 25-mL sterile pipettes
- Cryovials
- Hemacytometer and Trypan blue
- Complete Schneider's *Drosophila* Medium

  Note: Complete Schneider's *Drosophila* Medium contains 10% heat-inactivated fetal bovine serum (FBS). Pluronic F-68 should also be added at 0.1%, if culturing suspension cells.
- *Optional:* Penicillin-Streptomycin (Final concentration 50 units penicillin G and 50 µg streptomycin sulfate per milliliter of culture)
- 25-cm^2 flasks, 75-cm^2 flasks, and 35-mm plates (other flasks and plates may be used)

Initiate cell culture from frozen stock

The following protocol is designed to help you initiate a cell culture from a 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 100 × *g* for 5–10 minutes. Aseptically decant the medium containing DMSO and resuspend the cell pellet in 5 mL of fresh complete Schneider's *Drosophila* Medium.
4. Transfer the 5 mL of cell suspension into a T-25 cm^2 flask, and incubate in a 28°C non-humidified, ambient air-regulated incubator or warm room. Loosen caps of flasks to allow oxygenation/aeration.
5. Incubate at 28°C until cells reach a density of 0.6×10^7–2.0×10^7 cells/mL. This may take 3–4 days.

Passage S2 cells

Note: 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. 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.
2. 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 1:2 to 1:5 dilution into **new** culture vessels. Add complete Schneider's *Drosophila* Medium and incubate at 28°C incubator until the density reaches 0.6 × 10^6–2 × 10^6 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.

Note: 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.

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 1000 × *g* for 2–3 minutes in a table top centrifuge at 4°C. Reserve the conditioned medium.
4. Resuspend the cells in 10 mL PBS and pellet at 1000 × *g* for 2–3 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, or wrap vials in paper towels and place in a well-insulated container lined with additional paper towels. 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.

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.

Transfect S2 cells

Introduction

Drosophila Schneider 2 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 **www.lifetechnologies.com**.

Important

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.

Note

You may set up transient and stable transfections in side-by-side experiments for efficiency. If expression is detected from the transient transfection, you may proceed directly with selection of polyclonal cell lines.

Selection vector

The DES™ kits are available with a choice of pCoHygro or pCoBlast selection vectors (see page iv for ordering information). 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 guidelines

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 starting

Be sure and 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 provided for transient and stable transfections. 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

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

   In a microcentrifuge tube mix together the following components. This will be **Solution A**.

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 a final volume of 300 µL

4. 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**.
5. 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.
6. Incubate the resulting solution at room temperature for 30–40 minutes. After ~30 minutes a fine precipitate should form.
7. Mix the solution and add dropwise to the cells. Swirl to mix in each drop.
8. 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)

If you are performing a transient transfection, continue with the steps 9–11. If you are selecting stable transfectants, proceed to **Calcium phosphate transfection (stable)**.

Day 3: Post-transfection (transient expression)

9. Remove calcium phosphate solution and wash the cells twice with complete medium. Add fresh, complete Schneider's *Drosophila* Medium and replate into the same vessel. Continue to incubate at 28°C.
10. **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. 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)

11. Harvest the cells 2, 3, 4, and 5 days posttransfection and assay for expression of your gene (see page 11). There is no need to add fresh medium or additional inducer.

Calcium phosphate transfection (stable)

Day 3: Post-transfection (stable transfection)

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

Day 5: Selection (stable transfection)

11. Centrifuge cells and resuspend in complete Schneider's *Drosophila* Medium containing the appropriate selection agent. 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)

12. Centrifuge cells and resuspend in complete Schneider's *Drosophila* Medium containing the appropriate selection agent. 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.
13. Expand resistant cells into 6-well plates to test for expression (see **Testing for Expression**, page 11) or into flasks to prepare frozen stocks (page 7). **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. 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. 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

Troubleshooting

Use the following table to troubleshoot any problem you might have with S2 cells.

Problem	Solutions
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. If cells do not seem to be growing, replate new cells.
	Cells grow better if conditioned medium is brought along during passage.
Low Transfection Efficiency	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 **www.lifetechnologies.com**. (**Lipid-Mediated Transfection**).
Low or No Protein Expression	If using a secretion vector, gene was not cloned in-frame with signal sequence. If your protein is not in frame with the signal sequence, it will not be expressed or secreted.
	No Kozak sequence for proper initiation of transcription. Translation will be inefficient and the protein will not be 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.

Using different inducers

Other researchers have used 10 µM $CdCl_2$ to induce the metallothionein promoter (Johansen et al., 1989). 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 proteins (Millar et al., 1994; Tota et al., 1995; Wang et al., 1993).

Important

Remember to prepare master stocks and working stocks of your stable cell lines prior to scale-up and purification.

Appendix

Technical support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited product warranty

Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

References

Han, K. (1996). An Efficient DDAB-Mediated Transfection of *Drosophila* S2 Cells. Nucleic Acids Res. *24*, 4362-4363.

Johansen, H., van der Straten, A., Sweet, R., Otto, E., Maroni, G., and Rosenberg, M. (1989). Regulated Expression at High Copy Number Allows Production of a Growth Inhibitory Oncogene Product in *Drosophila* Schneider Cells. Genes and Development *3*, 882-889.

Millar, N. S., Buckingham, S. D., and Sattelle, D. B. (1994). Stable Expression of a Functional Homo-Oligomeric *Drosophila* GABA Receptor in a *Drosophila* Cell Line. Proc. R. Soc. Lond. B *258*, 307-314.

Schneider, I. (1972). Cell Lines Derived from Late Embryonic Stages of *Drosophila melanogaster*. J. Embryol. Exp. Morph. *27*, 363-365.

Tota, M. R., Xu, L., Sirotina, A., Strader, C. D., and Graziano, M. P. (1995). Interaction of [flourescein-Trp25]Glucagon with Human Glucagon Receptor Expressed in *Drosophila* Schneider 2 Cells. J. Biol. Chem. *270*, 26466-26472.

Wang, W.-C., Zinn, K., and Bjorkman, P. J. (1993). Expression and Structural Studies of Fasciclin I, an Insect Cell Adhesion Molecule. J. Biol. Chem. *268*, 1448-1455.