Drosophila Schneider 2 (S2) Cells

Catalog no. R690-07

Version F
050202
28-0172

www.invitrogen.com
tech_service@invitrogen.com

Table of Contents

Important Information

Shipping/Storage

Shipping:

- Cells are shipped on dry ice.

Storage: Upon receipt--

- Store the cells in liquid nitrogen

Kit Contents

One vial of Schneider 2 (S2) cells is supplied (1 ml per vial, 1 x 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 Invitrogen.

Product	Amount	Catalog no.
Schneider's *Drosophila* Medium	500 ml	11720-034
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

Product Qualification

The following criteria are used to qualify S2 cells:

- Cells are tested independently and certified to be free of mycoplasma.
- Prior to freezing, cells are greater than 95% viable. Forty-eight hours after thawing, cells are greater than 90% viable.

Methods

Culturing 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 room temperature 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 below 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 28°C incubator and do not require CO_2. **Note:** If you want to slow down S2 cell growth, you may incubate cells at room temperature (22-25°C).
- The complete medium for S2 cells is Schneider's *Drosophila* Medium containing 10% **heat-inactivated** FBS. This medium is used for transient expression and stable selection. Schneider's *Drosophila* Medium is available separately from Invitrogen (Catalog no. 11720-034). Heat-inactivated FBS must be added to a final concentration of 10% before use.
- **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, pass S2 cells when cell density is between 6 to 20 x 10^6 cells/ml and split at a 1:2 to 1:5 dilution. **Note**: S2 cells do not grow well when seeded at a density below 5 x 10^5 cells/ml.

  For example, transfer 2 ml of a 10 ml cell suspension at 2.0 x 10^7 cells/ml to a new 75 cm^2 flask containing 10 ml of new medium.
- S2 cells grow better if some conditioned medium is brought along when passaging cells. **Note:** Conditioned medium is medium in which cells have been grown.

Important

S2 cells do not completely adhere to surfaces, making it difficult to rinse the cells if needed. To exchange cells into new medium or to wash cells prior to lysis, follow the instructions below:

- Resuspend cells in the conditioned medium and centrifuge at 1000 x g for 2 to 3 minutes. Decant the medium.
- Resuspend the cells in fresh medium (or PBS) and centrifuge as above. Repeat.
- Add fresh medium (or buffer) and replate the cells (or lyse them).

continued on next page

Culturing S2 Cells, continued

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 (contains 10% heat-inactivated fetal bovine serum (FBS))
- Optional: Penicillin-Streptomycin (Final concentration 50 units penicillin G and 50 µg streptomycin sulfate per milliliter of culture)
- Table-top centrifuge
- 25 cm^2 flasks, 75 cm^2 flasks, and 35 mm plates (other flasks and plates may be used)
- Phosphate-Buffered Saline (PBS; available from Gibco™, Catalog no. 10010-023)

Initiating 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 x 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.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 25 cm^2 flask containing 5 ml of room temperature complete Schneider's *Drosophila* Medium.
3. Incubate at 28°C for 30 minutes.
4. Resuspend the cells and centrifuge at 1000 x g. Decant the medium to remove the DMSO and plate the cells in 5 ml fresh complete Schneider's *Drosophila* Medium.
5. Incubate at 28°C until cells reach a density of 6 to 20 x 10^6 cells/ml. This may take 3 to 4 days.

Passaging the S2 Cells

Note: Cells will start to clump at a density of ~5 x 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 to 4 x 10^6 cells/ml. Do not split cells below a density of 0.5 x 10^6 cells/ml.

   For example, 2 ml of cells from a 75 cm^2 flask at a density of 2 x10^7 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. Proceed to next page.

continued on next page

Culturing S2 Cells, continued

Passaging the S2 Cells, continued

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 6 to 20 x 10^6 cells/ml.
5. Repeat Steps 1-4 as necessary to expand cells for transfection or expression.

Freezing 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 1.0-2.0 x 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 x g for 2 to 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 x g for 2 to 3 minutes.
5. Prepare Freezing Medium (see recipe above).
6. Resuspend the cells at a density of 1.1 x 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.

Transfecting 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 (see page 8).

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 to 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 as described below.

- 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).

continued on next page

Transfection of S2 Cells, continued

Before Starting

Be sure and have the following reagents and equipment ready before starting:

- S2 cells growing in culture (3 x 10^6 S2 cells per 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, Catalog no. K2780-01)

Calcium Phosphate Transfection

Instructions are included below and on the next page for transient and stable transfections. Instructions are for one transfection in a 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 x 10^6 S2 cells (1 x 10^6 cells/ml) in a 35 mm plate in 3 ml complete Schneider's *Drosophila* Medium.
2. Grow 6 to 16 hours at 28°C until cells reach a density of 2 to 4 x 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 to 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 to 24 hours at 28°C. **Note**: You may wish to investigate whether extending the incubation time improves transfection efficiency.

continued on next page

Transfection of S2 Cells, continued

Calcium Phosphate Transfection (Transient)

If you are performing a transient transfection, continue with the steps below. If you are selecting stable transfectants, proceed to the next section.

Day 3: Posttransfection (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 (2-4 x 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+: Harvesting Cells (Transient Expression)

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

Calcium Phosphate Transfection (Stable)

Day 3: Posttransfection (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 to 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 to 20 x 10^7 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 next page) or into flasks to prepare frozen stocks (page 3). **Always use complete Schneider's *Drosophila* Medium containing the appropriate concentration of selection agent when maintaining stable S2 cell lines.**

continued on next page

Transfection of S2 Cells, continued

Testing 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 x g for 2 to 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 to 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

Use the table below 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 x 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 (Catalog 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 (see next page).

continued on next page

Troubleshooting, continued

Problem	Solutions
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.

Lipid-Mediated Transfection

S2 cells may also be transfected using some lipid-based transfection reagents including Cellfectin® Reagent available from Invitrogen (Catalog no. 10362-010) and dimethyldioctadecylammonium bromide (DDAB) (Han, 1996). For more information about Cellfectin® Reagent, contact Technical Service (see page 9).

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 Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

Japanese Headquarters:
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tel (Toll Free): 0800 5345 5345
Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, please visit our web site (www.invitrogen.com) and follow the instructions below.

1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

continued on next page

Technical Service, continued

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

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.