USER GUIDE

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Growth and Maintenance of the GripTite™ 293 MSR Cell Line

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For Research Use Only. Not for diagnostic procedures.

life technologies™

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Kit Contents and Storage

Shipping and Storage

The GripTite™ 293 MSR Cell Line and other components supplied with the cell line are shipped and should be stored as detailed below.

Item	Shipping	Storage
GripTite™ 293 MSR Cell Line	Dry ice	Liquid nitrogen
Versene	Dry ice	+4°C
Geneticin®	Dry ice	+4°C

Kit Contents

The following reagents are supplied with the GripTite™ 293 MSR Cell Line. Store as directed.

Reagent	Composition	Amount	Storage
GripTite™ 293 MSR Cell Line	Each vial contains 3×10^6 cells in 1 ml of Freezing Medium	2 vials	Liquid nitrogen
Versene 1:5000 (1X), liquid	0.2 g/L EDTA•4Na in phosphate-buffered saline	100 ml	+4°C
Geneticin® Selective Antibiotic, liquid	50 mg/ml active Geneticin® in distilled water	20 ml	+4°C

Handle as potentially biohazardous material under at least Biosafety Level 2 containment. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

Product Use

For research use only. Not intended for any human or animal diagnostic or therapeutic uses.

Accessory Products

Accessory Products

Some of the reagents supplied with the GripTite™ 293 MSR Cell Line as well as other products that may be used with the GripTite™ 293 MSR Cell Line are available separately from Life Technologies. For more information, refer to our website (**www.lifetechnologies.com**) or call Technical Support (see page15). **Note:** Some reagents are available in other sizes.

Item	Amount	Catalog no.
Versene 1:5,000, liquid	100 ml	15040-066
Geneticin®, liquid (50 mg/ml)	20 ml	10131-035
	100 ml	10131-027
Dulbecco's Modified Eagle Medium (D-MEM) (high glucose)	500 ml	11965-092
Fetal Bovine Serum	500 ml	26140-079
10 mM MEM Non-Essential Amino Acids Solution	100 ml	11140-050
Penicillin-Streptomycin	100 ml	15070-063
Trypsin-EDTA (0.25% Trypsin, 1 mM EDTA•4Na)	100 ml	25200-056
Advanced D-MEM Reduced Serum Medium	500 ml	12491-015
Dulbecco's Phosphate-Buffered Saline (D-PBS) (contains no calcium or magnesium)	500 ml	14190-144
Lipofectamine® 2000 Reagent	0.75 ml	11668-027
	1.5 ml	11668-019
Opti-MEM® I Reduced Serum Medium	100 ml	31985-062

Introduction

Overview

Introduction

The GripTite™ 293 MSR Cell Line is derived from the 293-H cell line (see the next page) and stably expresses the human macrophage scavenger receptor type 1 (*MSR I*), class A gene (Matsumoto *et al.*, 1990; Robbins and Horlick, 1998) from the pCMV SPORT6 MSR.neo plasmid. Expression of the macrophage scavenger receptor is controlled by the human cytomegalovirus (CMV) promoter and is high-level and constitutive. For more information about pCMV SPORT6 MSR.neo, see the **Appendix**, page 14.

Features of the GripTite™ 293 MSR Cell Line

GripTite™ 293 MSR Cells exhibit the following features:

- Constitutively express high levels of the Macrophage Scavenger Receptor protein, resulting in greater adherence to tissue culture substrates when compared to parental 293-H cells (Cheung *et al.*, 1996; Robbins and Horlick, 1998)
- GripTite™ 293 MSR monolayer cultures can withstand multiple wash steps, making them ideal for use in high-throughput toxicological and drug screening assays, histochemical staining, or other anchorage-dependent cell culture applications (see page 12 for more information)
- Prepared from low passage Master Cell Bank cultures that are only 13 to 16 total passages
- Maintain adherent characteristics for at least 30 passages or 3 months when maintained in medium containing Geneticin®
- Demonstrate high transfection efficiencies using Lipofectamine® 2000 Reagent (see page 10 for more information), and high-level transgene expression

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Overview, continued

Parental Cell Lines

The 293 cell line is a permanent line established from primary embryonal human kidney transformed with sheared human adenovirus type 5 DNA (Graham *et al.*, 1977; Harrison *et al.*, 1977). The genes encoded by the E1 region of adenovirus (E1a and E1b) are expressed in these cells and participate in transactivation of some viral promoters, allowing these cells to produce very high levels of protein.

The 293-H cell line available from Life Technologies (Catalog no. 11631-017), is a fast-growing variant of the 293 cell line, and was originally selected for its superior growth and transfection efficiency (Roy *et al.*, 1999).

Antibiotic Resistance

GripTite™ 293 MSR cells stably express the neomycin resistance gene from pCMV SPORT6 MSR.neo and should be maintained in medium containing Geneticin® at the recommended concentration (see the next page). Expression of the neomycin resistance gene in GripTite™ 293 MSR cells is controlled by the SV40 enhancer/promoter. Geneticin® is included with the GripTite™ 293 MSR Cell Line and is also available separately from Life Technologies (see page vi for ordering information).

Media Requirements

Media for GripTite™ 293 MSR Cells

The table below lists the recommended complete medium and freezing medium required to maintain and culture the GripTite™ 293 MSR Cell Line (see page vi for information to order cell culture reagents). Note that GripTite™ 293 MSR cells should be maintained in complete media containing the indicated concentration of Geneticin®. Geneticin® is supplied with the GripTite™ 293 MSR Cell Line, but is also available separately from Life Technologies (see page vi for ordering information).

Complete Medium	Antibiotic	Freezing Medium
D-MEM (high glucose) 10% Fetal Bovine Serum (FBS) 0.1 mM MEM Non-Essential Amino Acids (NEAA)	600 µg/ml Geneticin®	90% complete medium 10% DMSO

General Guidelines

- FBS does not need to be heat-inactivated for use with the GripTite™ 293 MSR Cell Line.
- Antibiotics are not recommended; however, 5 ml/L of Penicillin/Streptomycin (Catalog no. 15070-063) may be used when required.

Reduced Serum Media

For applications where the presence of serum is a concern, it is possible to culture GripTite™ 293 MSR cells in reduced serum medium. We recommend using Advanced D-MEM (Catalog no. 12491-015) containing 2% FBS. Note however, that GripTite™ 293 MSR cells cultured in reduced serum media adhere less tightly to the tissue culture dish than GripTite™ 293 MSR cells cultured in D-MEM containing 10% FBS.

Serum-Free Media

Culturing GripTite™ 293 MSR cells in serum-free formulations such as 293 SFM II or CD 293 Medium is **not** recommended as cells lose their enhanced adherent properties.

Methods

General Information

General Cell Handling Guidelines

Follow the general guidelines below to grow and maintain GripTite™ 293 MSR cells.

- **Make sure that all solutions and equipment that come in contact with the cells are sterile.** Always use proper sterile technique and work in a laminar flow hood.
- Before starting experiments, be sure to have cells established and also have some frozen stocks on hand. We recommend using early-passage cells for your experiments.
- For general maintenance of cells, pass GripTite™ 293 MSR cells as recommended (see **Subculturing Cells**, page 6). Avoid overgrowing cells before passaging.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.
- Cells should be at the appropriate confluence and at >85% viability prior to transfection (see page 10).

Before Starting

Have the following solutions and supplies on hand:

- 15 ml sterile, conical tubes
- Appropriate sized tissue culture flasks and pipettes
- Cryovials
- Dulbecco's Phosphate-Buffered Saline (D-PBS; Catalog no. 14190-144)
- Versene (supplied with the cells)
- 0.25% Trypsin, 1 mM EDTA•4Na solution (Catalog no. 25200-056)
- Complete medium
- 50 mg/ml Geneticin® (supplied with the cells)
- Reagents for counting cells
- Table-top centrifuge

Thawing Cells

Introduction

Two vials of GripTite™ 293 MSR cells are provided, with each vial containing 3×10^6 cells in 1 ml of Freezing Medium. We recommend thawing 1 vial of cells, and retaining the second vial as a back-up for future use. Store frozen GripTite™ 293 MSR cells in liquid nitrogen until ready to use.

Thawing Procedure

Use the following procedure to thaw GripTite™ 293 MSR cells to initiate cell culture. Thaw cells into prewarmed, complete medium **without** Geneticin®.

1. Remove the frozen vial of cells from liquid nitrogen and thaw quickly in a 37°C water bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol.
3. Triturate and transfer the entire contents of the cryovial into a T-75 cm^2 flask containing 17 ml of pre-warmed, complete medium without Geneticin®.
4. Incubate the cells overnight in a 37°C incubator containing a humidified atmosphere of 5-8% CO_2 in air. Loosen caps of flasks to allow oxygenation/aeration.
5. The following day, replace the medium with 15 ml of prewarmed, complete medium containing 600 µg/ml Geneticin®.
6. Incubate the cells and check them daily until the cells are 80-90% confluent.
7. Proceed to **Subculturing Cells**, next page.

We recommend subculturing cells for a minimum of 3 passages after thawing before use in other applications.

Subculturing Cells

Introduction

Follow the recommendations and procedures in this section to subculture GripTite™ 293 MSR cells. Maintain cells as adherent monolayer cultures in complete medium containing 600 µg/ml Geneticin®.

Important

Because of their adherent properties, we recommend treating GripTite™ 293 MSR cells with Versene as well as trypsin-EDTA to remove cells from tissue culture plates (see procedure on page 8). Using this procedure, GripTite™ 293 MSR cells are easily detached. Versene is supplied with the GripTite™ 293 MSR Cell Line, but is also available separately from Life Technologies (see page vi for ordering information).

Note: GripTite™ 293 MSR cells will not detach from tissue culture surfaces when treated for 1-2 minutes with trypsin-EDTA alone. However, cells will detach if treated with trypsin-EDTA alone for 5-8 minutes at 37°C. Use of this method is **not** recommended as cells will exhibit reduced viability.

Note

GripTite™ 293 MSR cells may exhibit reduced viability during subculturing (>85% viability compared to >95% observed with parental 293-H cells) due to the shear forces required to remove cells from the plate.

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Subculturing Cells, continued

Subculturing Conditions

Use the following recommended conditions to subculture GripTite™ 293 MSR cells. For a procedure to subculture cells, see the next page.

Parameter	Recommended Condition
Cell density	>5 × 10^5 viable cells/ml (>80% confluent)
Culture vessel	T-75 cm^2 to T-162 cm^2 disposable sterile T-flasks. Dilute cells in a total working volume of 15-20 ml for T-75 cm^2 flasks and 40-50 ml for T-162 cm^2 flasks.
Seeding density	2 to 5 × 10^4 viable cells/cm^2
Incubation conditions	37°C incubator with a humidified atmosphere of 5-8% CO_2 in air; loosen caps to allow for oxygenation/aeration

Determining Viability and Cell Density

Follow the procedure below to determine viable and total cell counts.

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.
2. Determine viability and the amount of cell clumping using the trypan blue exclusion method.
3. Vigorously vortex cells for up to 40 seconds to break up cell clumps.
4. Determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

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Subculturing Cells, continued

Subculturing Procedure

Use this procedure to subculture GripTite™ 293 MSR cells grown in a T-75 cm^2 flask. If you are using other-sized flasks, scale the reagent volumes up or down accordingly.

1. Remove media from the flask. Rinse the flask with 5 ml of Dulbecco's Phosphate-Buffered Saline (D-PBS) without Ca^{2+} or Mg^{2+} and remove.
2. Add 1 ml of pre-warmed Versene to the flask, and incubate for 5 minutes at room temperature.
3. Remove Versene and add 1 ml of pre-warmed, 0.25% trypsin-1 mM EDTA solution to the flask. Incubate at 37°C until cells have detached (about 2 minutes). Check the cells under a microscope and confirm that most of the cells have detached. If cells are still attached, incubate a little longer until most of the cells have detached.
4. Add 4 ml of complete medium containing 600 µg/ml Geneticin® to the flask and transfer the cell suspension to a 15 ml sterile, conical tube.
5. Determine viable and total cell counts (see procedure on the previous page)
6. Seed cells at the recommended density (see table on the previous page), diluting in pre-warmed complete medium containing 600 µg/ml Geneticin®. Incubate flasks as recommended (see table on the previous page).

Freezing Cells

Introduction

When freezing GripTite™ 293 MSR cells, we recommend the following:

- Freeze cells at a density of **at least** 3 × 10^6 viable cells/ml.
- Use a freezing medium composed of 90% complete medium and 10% DMSO. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and store at +4°C until use. Discard any remaining freezing medium after use.

Freezing Procedure

Use the procedure below to freeze cells.

1. Culture the desired quantity of GripTite™ 293 MSR cells to 70-90% confluency.
2. Remove the cells from the tissue culture flask(s) following Steps 1-4, **Subculturing Procedure**, page 8.
3. Determine viable and total cell counts (see procedure on page 7) and calculate the volume of freezing medium required to yield a final cell density of ≥3 × 10^6 cells/ml.
4. Prepare the required volume of freezing medium (see above).
5. Centrifuge the cell suspension (from Step 2) at 100 × g for 5 to 10 minutes. Aseptically decant supernatant and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.
6. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications.
7. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
8. Transfer vials to liquid nitrogen storage.

Note: You may check the viability and recovery of frozen cells 24 hours after storing vials in liquid nitrogen by following the procedure outlined in **Thawing Procedure**, page 5.

Transfecting Cells

Introduction

GripTite™ 293 MSR cells are suitable for use in transient transfection studies or to generate stable cell lines. General guidelines for transfection are provided below.

Transfection Reagent

GripTite™ 293 MSR cells are amenable to transfection using standard methods including lipid-mediated transfection (Felgner *et al.*, 1989; Felgner and Ringold, 1989), calcium phosphate precipitation (Chen and Okayama, 1987; Wigler *et al.*, 1977), and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988).

For highest transfection efficiency, we recommend using the cationic lipid-based transfection reagent, Lipofectamine® 2000, available from Life Technologies (see page vi). Refer to the manual accompanying the product for instructions. Other transfection reagents are suitable.

Note: Using Lipofectamine® 2000, GripTite™ 293 MSR cells transfect with an efficiency similar to that observed with parental 293-H cells.

Transient Transfection

The GripTite™ 293 MSR Cell Line may be transiently transfected with any plasmid DNA. General guidelines are provided below.

- Make sure that cells are healthy at the time of plating. Overgrowth of cells prior to passaging can compromise their transfection efficiency.
- On the day before transfection, plate cells in complete medium without Geneticin® as recommended by the manufacturer of the transfection reagent you are using. **Note:** If you are using Lipofectamine® 2000, plate cells such that they will be approximately 90-95% confluent at the time of transfection.
- Transfect your plasmid construct into GripTite™ 293 MSR cells using the method of choice (see above).
- Allow the cells to recover for 24-48 hours before proceeding to assay for expression of your gene of interest.

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Transfecting Cells, continued

Generating Stable Cell Lines

GripTite™ 293 MSR cells can be used as hosts to generate a stable cell line expressing your gene of interest from most plasmids. To generate a stable cell line:

1. One day before transfection, plate cells in complete medium without Geneticin® as recommended by the manufacturer of the transfection reagent you are using.
2. Transfect your plasmid construct into GripTite™ 293 MSR cells using the method of choice (see the previous page).
3. Allow the cells to recover for 24 hours, then trypsinize cells following Steps 1-4, **Subculturing Procedure**, page 8, and split the cells into complete medium containing 600 µg/ml Geneticin®.
4. The following day, replace the medium with Selection Medium containing 600 µg/ml Geneticin® **and** the appropriate additional selective antibiotic.
5. Replace the Selection Medium every 3 to 4 days until discrete colonies become visible.
6. Pick colonies and expand the cells to test for expression of the gene of interest.

High-Throughput Applications

Introduction

GripTite™ 293 MSR cells are ideal for use in high-throughput (HTP) applications where adherence to tissue culture surfaces is important, such as procedures requiring washing or staining steps. In these procedures, parental 293-H cells are easily dislodged, while GripTite™ 293 MSR cells remain attached to the plastic. General recommendations are provided in this section to use GripTite™ 293 MSR cells for HTP applications.

High-Throughput Applications

GripTite™ 293 MSR cells adhere well to tissue culture surfaces under the following conditions:

- Standard staining procedures (*e.g.* staining for β-galactosidase activity)
- Washing with a 12-channel pipettor
- Washing with commercially available plate washers including the CCS Packard PlateWash™, Molecular Devices Embla 96/384 Well Washer, Tecan PW-96, and ASYS Hitech FlexiWash I.
- Manipulation with a liquid handling robot

Using Plate Washers

For assays where plate washing is required, you may use either slant-pin or straight-pin dispensing heads when performing wash steps with commercial plate washers. Use of straight-pin dispensing heads results in reproducible protein recovery from plates; however, because of the extreme shear forces, some loss of cells may be noted directly below the dispensing pin. For a recommendation to address this issue, see **General Guidelines for HTP Applications**, next page. Note that this cell loss is not observed when using slant-pin dispensing heads for plate washing.

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High-Throughput Applications, continued

General Guidelines for HTP Applications

Follow the general guidelines below when using GripTite™ 293 MSR cells for HTP applications.

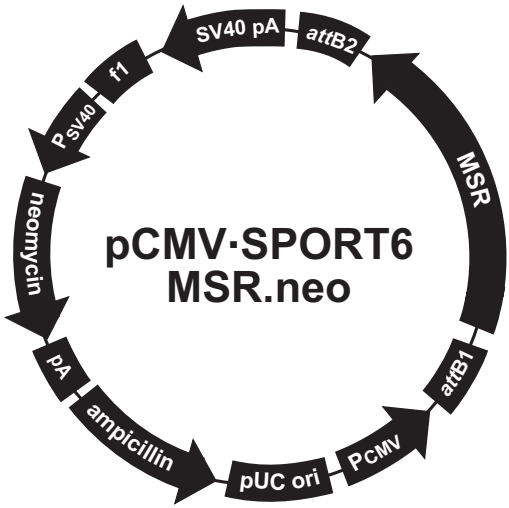
- After plating, allow cells to attach overnight before use to guarantee adherence.
- **Do not** allow cells to become overly confluent before performing assays. Once confluent, cells will start to adhere to each other and become clumpy. Clumped cells are more likely to float off the dish during the assay.
- Incubate cells for as long as possible in a 37°C incubator with a humidified atmosphere of 5-8% CO_2 in air before performing the assay. Do not keep cells for an extended period of time in a non-CO_2 atmosphere.
- When using a straight-pin dispensing head in a plate washer, position the head as high as possible above the well and in such a way that the liquid dispenses against the side of the well. This minimizes loss of cells during the washing steps that are due to shear forces.
- Take care that the dispensing head or aspiration head are not positioned too closely to the bottom of the well. If the heads scrape the wells, cells will be removed.

Appendix

Map of pCMV·SPORT6 MSR.neo

Description

The figure below shows the pCMV•SPORT6 MSR.neo plasmid, which was stably integrated into 293-H cells to generate the GripTite™ 293 MSR Cell Line. The plasmid uses the human cytomegalovirus (CMV) immediate early promoter to control expression of the Macrophage Scavenger Receptor (*MSR1*) gene.



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Purchaser Notification

Information for European Customers

The GripTite™ 293 MSR Cell Line is genetically modified and includes human adenovirus type 5 DNA and the pUC-derived plasmid, pCMV•Sport6 MSR.neo. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

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Notes

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

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www.lifetechnologies.com/support or email techsupport@lifetech.com

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