# R-Spondin1 Expressing 293T Cell Line

Immortalized Cell Line

**Cat. # SCC111**

**Pack size: ≥1X10$^6$ viable cells/vial**

Store in liquid nitrogen

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

**Data Sheet**



## Background

3D organoid culture systems are increasingly employed as powerful tools for the study of human diseases. Organoids are thought to better represent the tissue-specific niche microenvironment. R-spondin-1 (RSPO1) is one of the most extensively used niche factors for culturing 3D organoids. The first published report of an organoid culture system able to promote long-term survival and multilineage differentiation of intestinal stem cells (ISCs) employed mouse Rspo1 as one of the critical factors.[2] Since then, R-Spondin1 has been used to establish organoid cultures from the stomach, small intestine, colon, pancreas and liver from both mouse and human sources [1, 3].

RSPO1 is an intestinal growth factor that works as a potent activator of the Wnt/beta-catenin signaling pathway by binding to leucine-rich repeat–containing G protein–coupled receptors (LGRs), which are expressed in tissue stem cells. RSPO1 also binds to the transmembrane E3 ubiquitin ligases RNF43 and ZNRF3 and triggers their internalization, thereby potentiating Wnt signaling.

The cell line is a transfectant of 293T cells expressing mouse R-spondin1 protein tagged with N-terminus HA and C-terminus Fc. R-Spondin1 Expressing 293T cell line can be used to produce either purified Rspo1 or Rspo1 conditioned media. The FC region and HA tags enable ease of purification and characterization.

## Short Tandem Repeat (STR) Profile

| | |
|---|---|
| D3S1358: 16, 17 | D16S539: 9, 14 |
| TH01: 7, 9.3 | CSF1PO: 11, 12 |
| D21S11: 28, 29, 30, 30.2 | Penta D: 9, 10 |
| D18S51: 17, 18, 19 | vWA: 16, 19, 20 |
| Penta E: 7, 15 | D8S1179: 11, 12, 13, 14 |
| D5S818: 7, 8, 9 | TPOX: 11 |
| D13S317: 12, 14 | FGA: 22, 23 |
| D7S820: 11 | Amelogenin: X |

Immortalized cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

## Quality Control Testing

- Each vial contains ≥ 1X10$^6$ viable cells.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, B, C, Herpesvirus type 6, 7, 8 and HIV-1 & 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

## Storage and Handling

R-Spondin1 Expressing 293T Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

## Data

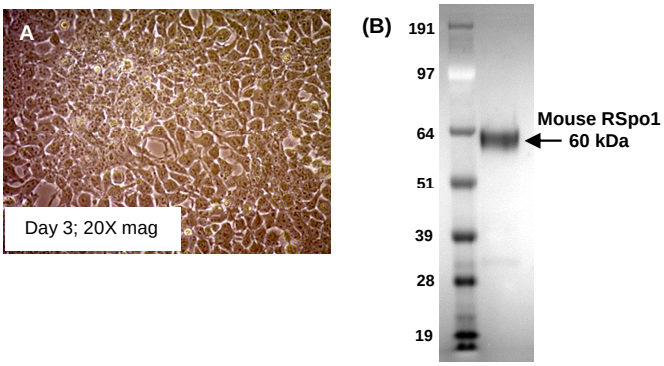


**Figure 1:** Day 3 culture of R-Spondin1 expressing 293T cells (**A**). 60 kDa Mouse R-Spondin1-Fc protein was purified by Protein A affinity purification (**B**). Purity: >95% SDS-PAGE visualized with quantitative densitometry by Coomassie Blue staining. Approximately 12 µg of protein was purified from 75 mL of FC-RSpo1-HA expressing 293T cell supernatant.

## Related References

1. Sato T, Clevers H (2015) Cell 161(7): 1700-1700.e1.
2. Ootani A, et al. (2009) Nat Med 15(6): 701-706.
3. Sato T, Clevers H (2013) Science 340(6137): 1190-1194.

**SPECIES LEGEND:** **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

**Please visit www.millipore.com for additional product information and references.**
Submit your published journal article, and earn credit toward future purchases. Visit www.millipore.com/publicationrewards to learn more!

Rev. 2.0/2018-03-13/SCC111/AN

## Protocols

### Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in DMEM-High Glucose (Sigma Cat. No. D6546), 10% FBS (EMD Millipore Cat. No. ES-009-B), 2 mM L-Glutamine (EMD Millipore Cat. No. TMS-002-C) and 1X Penicillin-Streptomycin Solution (EMD Millipore Cat. No. TMS-AB2-C).

2. Remove the vial of frozen R-Spondin1 Expressing 293T cells from liquid nitrogen and incubate in a 37℃ water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   **IMPORTANT: Do not vortex the cells.**

3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of R-Spondin1 Expressing 293T Expansion Medium (Step 1 above) to the 15 mL conical tube.

   **IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.**

6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   **IMPORTANT: Do not vortex the cells.**

7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 10-15 mL of R-Spondin1 Expressing 293T Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37℃ in a humidified incubator with 5% $CO_2$.
12. The next day, exchange the medium with 10-15 mL of fresh R-Spondin1 Expressing 293T Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 90-95% confluent, they can be dissociated with Accutase (EMD Millipore Cat. No. SCR005) or trypsin-EDTA (EMD Millipore Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use. Typical split ratio is 1:6 to 1:10.

### Purification of FC-RSpo1-HA Protein

Below is a protocol for a small scale (75mL) purification. Scale up accordingly.

1. Culture R-Spondin1 Expressing 293T cells until cells are 90-95% confluent.
2. Replace with CD293 Medium (Fisher Cat. No. 11913-019) containing L-glutamate for 7-14 days without medium change.
3. Collect the supernatant and centrifuge at 3000 rpm at 2-8°C for 10 – 15 minutes to remove cellular debris.
4. Collect the supernatant and filter using 0.22 µm filter.
5. Incubate filtered supernatant with pre-washed (with 1X PBS) Protein A agarose beads (Sigma Cat. No. P9424) for 3 hours at 2-8°C.
6. Collect the beads by centrifugation and transfer to a spin column (Pierce Cat. No. 69705).
7. Wash beads four times with 1X PBS.
8. Elute protein with 100mL and then 50 mL (total 150 mL) of Elution Buffer (0.2M Glycine, pH 2.8) and mixed with 150 mL of Notarization buffer (1M Tris HCL, pH 8.5).

   **Note:** From 1L of SNT, you can get around 2 mg of RSpo1 protein.

9. Dialyze with 1L of PBS with 10% glycerol twice using the Slide-A-Lyzer 10K Dialysis cassettes at 2-8°C (Fisher Cat. No. 66380).
10. Aliquot purified protein to avoid repeated freeze-thaw cycles. Freeze protein aliquots with liquid nitrogen (snap frozen) and store at -80°C.
11. Check the purified protein by Coomassie & Bradford protein assay

### Cryopreservation of Cells

R-Spondin1 Expressing 293T Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

## RESTRICTED USE AGREEMENT
**(subject to local law)**

**THIS PRODUCT MAY ONLY BE USED FOR RESEARCH PURPOSES, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS RESTRICTED USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL MILLIPORESIGMA ("SELLER") CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.**

"Product" means R-Spondin1 Expressing 293T Cell Line (SCC111)

"Research Purposes" means any internal *in vitro* research use and specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to PURCHASER's officers, employees, and students who need to use the Product for Research Purposes. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These restrictions will remain in effect for as long as PURCHASER possesses the Product.

**PLEASE CONTACT licensing@emdmillipore.com PRIOR TO PURCHASE FOR ANY USE OF THE PRODUCT OUTSIDE OF THIS RESTRICTED USE AGREEMENT.**

**GMO**
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.



**Please visit www.millipore.com for additional product information, test data and references**
EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502