R-Spondin1 Expressing 293T Cell Line

Immortalized Cell Line

Cat. # SCC111

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

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. 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.

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

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1358</td>
<td>16, 17</td>
</tr>
<tr>
<td>D21S11</td>
<td>28, 29, 30, 30.2</td>
</tr>
<tr>
<td>D18S11</td>
<td>17, 18, 19</td>
</tr>
<tr>
<td>Penta E</td>
<td>7, 15</td>
</tr>
<tr>
<td>D5S818</td>
<td>7, 8, 9</td>
</tr>
<tr>
<td>D13S317</td>
<td>12, 14</td>
</tr>
<tr>
<td>D7S820</td>
<td>11</td>
</tr>
<tr>
<td>D16S539</td>
<td>9, 14</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>11, 12</td>
</tr>
<tr>
<td>Penta D</td>
<td>9, 10</td>
</tr>
<tr>
<td>vWA</td>
<td>16, 19, 20</td>
</tr>
<tr>
<td>D8S1179</td>
<td>11, 12, 13, 14</td>
</tr>
<tr>
<td>D7S820</td>
<td>11</td>
</tr>
<tr>
<td>FGA</td>
<td>22, 23</td>
</tr>
<tr>
<td>TPOX</td>
<td>11</td>
</tr>
<tr>
<td>D8S1179</td>
<td>11, 12, 13, 14</td>
</tr>
<tr>
<td>Amelogenin</td>
<td>X</td>
</tr>
<tr>
<td>TH01</td>
<td>7, 9.3</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>11, 12</td>
</tr>
<tr>
<td>D18S11</td>
<td>17, 18, 19</td>
</tr>
<tr>
<td>Penta E</td>
<td>7, 15</td>
</tr>
<tr>
<td>D8S1179</td>
<td>11, 12, 13, 14</td>
</tr>
<tr>
<td>D7S820</td>
<td>11</td>
</tr>
<tr>
<td>FGA</td>
<td>22, 23</td>
</tr>
<tr>
<td>TPOX</td>
<td>11</td>
</tr>
</tbody>
</table>

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-RSp01-HA expressing 293T cell supernatant.

Related References

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°C 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. Transfer the cell mixture to a T75 tissue culture flask.

10. Incubate the cells at 37°C in a humidified incubator with 5% CO₂.

11. Incubate the cells at 37°C in a humidified incubator with 5% CO₂.

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.


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.
R-Spondin1 Expressing 293T Cell Line
Cat # SCC111

antibodies       Multiplex products         biotools      cell culture      enzymes      kits       proteins/peptides      siRNA/cDNA products

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

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures. Not for human or animal consumption. Purchase of this Product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.

Millipore®, the M mark, Upstate®, Chemicon®, Linco® and all other trademarks, unless specifically identified above in the text as belonging to a third party, are owned by Merck KGaA, Darmstadt. Copyright ©2008-2018 Merck KGaA, Darmstadt. All rights reserved.