

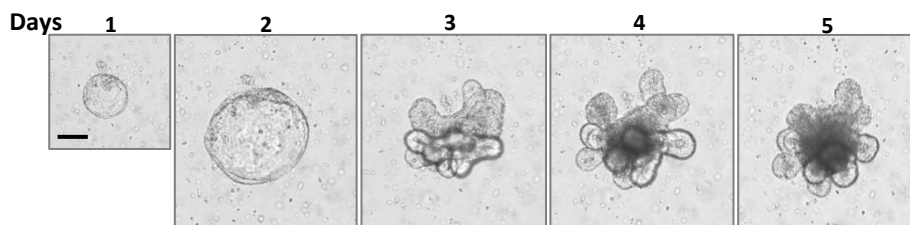


CULTREX® ORGANOID CULTURE

All Cultrex® Brand products are designed, developed and delivered by Trevigen, Inc.

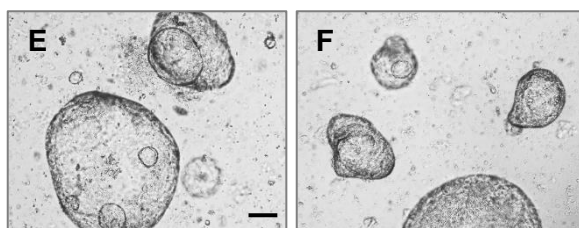
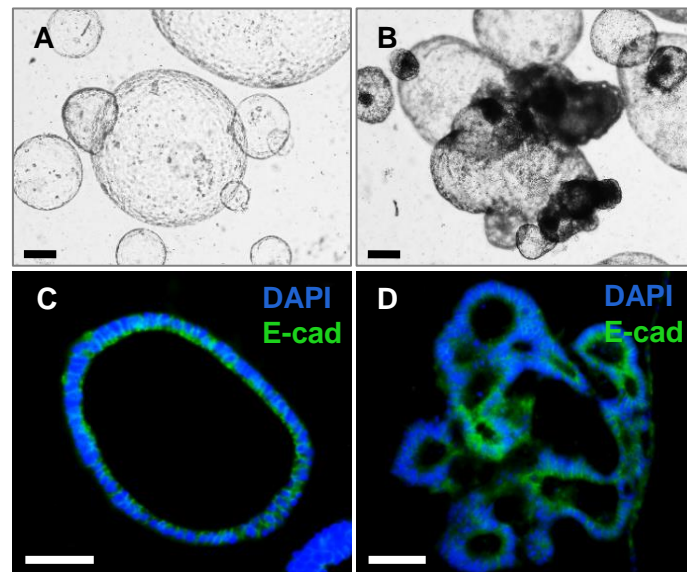
Organoid Qualified Matrices

Our exclusive organoid qualified matrices were designed and developed for expanding, passaging and differentiating cultures to support reproducible and robust organoid models. Products include two types of Basement Membrane Matrix (sold as Cultrex Basement Membrane Extract). The extensive organoid qualification that these products go through involves evaluation of take rate, proliferation and morphology of enteric organoids embedded in each BME. Cultrex Basement Membrane Extract type 2 and R1 are excellent choices to start and develop your organoid culture models. Cultrex RGF BME Type R1 provides a formulation that has higher concentration of entactin, one of the BME components that connects laminins and collagens reinforcing the hydrogel structure, when compared to Cultrex BME, Cultrex BME Type 2 and Cultrex BME Type 3. This formulation provides a more robust matrix for difficult to culture organoids. Cultrex RGF BME Type R1 has been specifically designed to culture tissue organoids, and is qualified using Small Intestine or Gastric organoid progenitor cells.



Growth of mouse colon organoids in Cultrex Reduced Growth Factor Type R1. Mouse colon organoids were grown embedded in Basement Membrane Matrix BME Type R1. Pictures show growth from day 1 to day 5 after passage. Scale bar: 100 µm.

Differentiation of Mouse small intestine organoids in Cultrex Reduced Growth Factor Type R1. Mouse small intestine organoids were grown from progenitor cells embedded in Basement Membrane Matrix BME Type R1. These organoids usually grow as spherical hollow structures (Panels A and C) but upon differentiation, crypt- and villi-like structures start to form (Panels B and D). Pictures A and D were taken as bright-field images, scale bar: 200 µm, whereas images C and D show immunofluorescent staining of E-cadherin (green). DAPI was used as a counterstain (blue). Scale bar: 50 µm.



Human Pancreatic Organoids embedded in Cultrex Reduced Growth Factor Type 2. Organoids originated from human pancreatic progenitor cells are shown growing embedded in Basement Membrane Matrix BME Type 2 (Images E and F). Scale bar: 200 µm.

Name	Catalog Number	Size
Cultrex® Reduced Growth Factor Basement Membrane Extract, Type R1	3433-005-R1*	5 ml
Cultrex® Basement Membrane Extract, Type 2	3532-005-02*	5 ml
Cultrex® Reduced Growth Factor Basement Membrane Extract, Type 2	3533-005-02*	5 ml

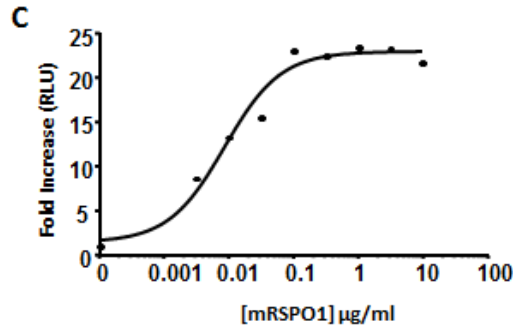
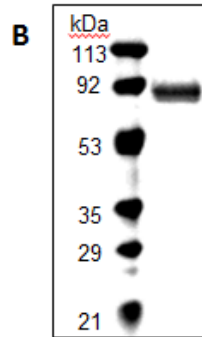
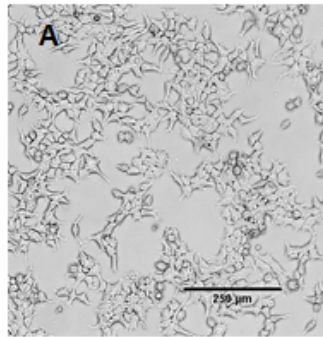
*10 ml and 1 ml sizes also available



R-spondin1 (Rspo1) Cells

The 293T cell line is stably transfected to express murine Rspo1 with an N-terminal HA epitope tag and fused to a C-terminal murine IgG2a Fc fragment. This cell line is used to produce either purified Rspo1 or Rspo1 conditioned media. The murine Rspo1 protein has been used extensively in organoid culture to maintain Lgr5+ stem cells in both murine and

human models, and the Fc and HA tags make it easy to purify or characterize.



Production of R-Spondin1 for organoid culture. A) The HA-R-Spondin1-Fc 293T cell line is cultured with Zeocin™ to select for

stably transfected cells. B) Production of HA-R-Spondin1-Fc is characterized using Western blot for R-Spondin1 protein (arrow). The relative molecular weight is approximately 70-75 kDa. C) HA-R-Spondin1-Fc induces activation of Wnt/β-catenin response when evaluated using the TopFlash Luciferase assay. Protocols on how to perform the Topflash assay are available at www.trevigen.com under Product Literature of Rspo1 cells.

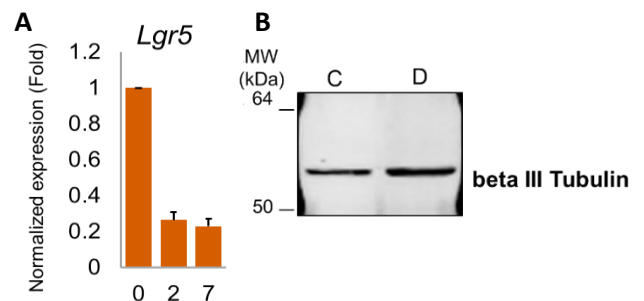
Note: Starting January 1st, 2017 free sample sizes of Cultrex Organoid Harvesting Solution, Cultrex RGF BME Type 2, and Cultrex RGF BME Type R1 will be added to your order of R-Spondin1 cells free of charge.

Name	Catalog Number	Size
Cultrex® R-spondin1 (Rspo1) Cells	3710-001-K	1 vial (0.5 ml), 1x10 ⁶ cells

Organoid Harvesting Solution

Cultrex Organoid Harvesting Solution provides a non-enzymatic method for depolymerizing extracellular matrix proteins to allow for harvesting of intact organoids for passaging, cryopreservation, or biochemical analysis. Cultrex Organoid Harvesting Solution is compatible with the main biochemical analysis techniques, such as quantitative PCR (qPCR) and western blotting. Protocols on how to harvest organoids and perform qPCR or western blot are available at www.trevigen.com in the Helpful Resources section.

Cultrex Organoid Harvesting Solution is compatible with biochemical analysis techniques. Mouse small intestine organoid pellets, harvested with Cultrex Organoid Harvesting Solution and resuspended in either TRIzol® (for total RNA extraction) or RIPA buffer (for total lysate preparation). Panel A shows a decrease in expression of Lgr5 (a stem cell marker in enteric cells) measured by qPCR after 0, 2 and 7 days of differentiation. In panel B a western blot against beta III Tubulin for control (C) and differentiated (D) organoids was performed.



Name	Catalog Number	Size
Cultrex® Organoid Harvesting Solution	3700-100-01	100 ml

Trevigen, Inc.

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