

## DESCRIPTION

**Source** *E. coli*-derived human Betacellulin/BTC protein  
Asp32-Gln118, with an N-terminal Met  
Accession # P35070.1  
Produced using non-animal reagents in an animal-free laboratory.

**N-terminal Sequence Analysis** Met-Asp32

**Predicted Molecular Mass** 9.9 kDa

## SPECIFICATIONS

**SDS-PAGE** 12-15 kDa, under reducing conditions.

**Activity** Measured in a cell proliferation assay using Balb/3T3 mouse embryonic fibroblast cells. Rubin, J.S. *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:415. The ED<sub>50</sub> for this effect is 0.100-1.50 ng/mL.

**Endotoxin Level** <0.10 EU per 1 µg of the protein by the LAL method.

**Purity** >97%, by SDS-PAGE with quantitative densitometry by Coomassie® Blue Staining.

**Formulation** Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

## PREPARATION AND STORAGE

**Reconstitution** Reconstitute at 100-500 µg/mL in PBS.

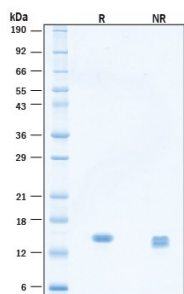
**Shipping** The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.

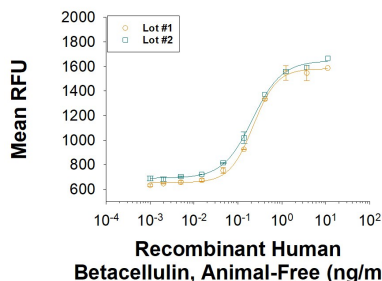
## DATA

### SDS-PAGE



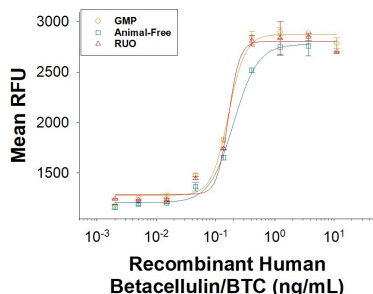
**Recombinant Human Betacellulin Protein, Animal-Free SDS-PAGE.** 2 µg/lane of Animal-Free™ Recombinant Human Betacellulin Protein (Catalog # BT-BTC-AFL) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® blue staining, showing bands at 12-15 kDa.

### Bioactivity



**Recombinant Human Betacellulin Protein, Animal-Free, Bioactivity.** Animal-Free™ Recombinant Human Betacellulin (Catalog # BT-BTC-AFL) as measured in a cell proliferation assay using Balb/3T3 mouse embryonic fibroblast cells. The ED<sub>50</sub> for this effect is 0.100-1.50 ng/mL. Two independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of Animal-Free Betacellulin.

### Bioactivity



**Equivalent Bioactivity of GMP, Animal-Free, and RUO grades of Recombinant Human Betacellulin/BTC.** Equivalent bioactivity of GMP (Catalog # BT-BTC-GMP), Animal-Free (Catalog # BT-BTC-AFL) and RUO (Catalog # BT-BTC) grades of Recombinant Human Betacellulin/BTC as measured in a cell proliferation assay using Balb/3T3 mouse embryonic fibroblast cells (orange, green, red, respectively).

**BACKGROUND**

Betacellulin (BTC) is a new member of the EGF family of cytokines that is comprised of at least ten proteins including EGF, TGF- $\alpha$ , amphiregulin, HB-EGF, and the various heregulins. All of these cytokines are synthesized as transmembrane precursors and are characterized by the presence of one or more EGF structural units in their extracellular domain. The soluble forms of these cytokines are released by proteolytic cleavage. BTC, a heparin-binding protein, was originally isolated from the conditioned media of mouse pancreatic beta tumor cells as a 32 kDa glycoprotein composed of 80 amino acid residues. The cDNA encoding human BTC was cloned from a human breast adenocarcinoma cell line (MCF-7) cDNA library. Human and mouse cDNAs encode BTC precursor proteins of 178 and 177 amino acid residues, respectively. At the amino acid sequence level, human BTC precursor protein exhibits 79% identity with that of the mouse BTC precursor. In a mouse cell line transfected with human BTC cDNA, three forms of soluble human BTC have been detected: the glycosylated, intact BTC composed of 80 amino acid residues, a truncated molecule lacking 12 amino acid residues from the amino terminus, and a second truncated molecule lacking 30 amino acid residues from the amino terminus. The biological activities of these BTC forms were shown to be identical. BTC can bind to the EGF receptor and is a potent mitogen for Balb/c 3T3 fibroblasts, retinal pigment epithelial cells and vascular smooth muscle cells.

**MANUFACTURING SPECIFICATIONS****Animal-Free Manufacturing Conditions**

Our dedicated controlled-access animal-free laboratories ensure that at no point in production are the products exposed to potential contamination by animal components or byproducts. Every stage of manufacturing is conducted in compliance with R&D Systems' stringent Standard Operating Procedures (SOPs). Production and purification procedures use equipment and media that are confirmed animal-free.

**Production**

- All molecular biology procedures use animal-free media and dedicated labware.
- Dedicated fermentors are utilized in committed animal-free areas.

**Purification**

- Protein purification columns are animal-free.
- Bulk proteins are filtered using animal-free filters.
- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

**Quality Assurance**

- Low Endotoxin Level.
- No impairment of biological activity.
- High quality product obtained under stringent conditions.
- For ex vivo research or bioproduction, [additional documentation](#) can be provided.

[Please read our complete Animal-Free Statement](#)