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# 1. General Information

## 1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	Transforming Growth Factor- $\beta$ 1, human (hTGF- $\beta$ 1)	<ul style="list-style-type: none"> <li>Solution, filtered through 0.2 <math>\mu</math>m pore size membrane.</li> <li>1 <math>\mu</math>g/ml solution in PBS (phosphate buffered saline) and 1 mg/ml BSA (bovine serum albumin).</li> </ul> <p><i>i</i> Purity of BSA: &gt;98%, endotoxin (LAL): &lt;1 EU/mg BSA.</p>	1 bottle, 1 ml

## 1.2. Storage and Stability

### Storage Conditions (Product)

The product is shipped on dry ice.

When stored at  $-15$  to  $-25^{\circ}\text{C}$ , the product is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	red	Transforming Growth Factor- $\beta$ 1, human (hTGF- $\beta$ 1)	Store in aliquots at $-15$ to $-25^{\circ}\text{C}$ . <b>⚠ Avoid repeated freezing and thawing.</b>

## 1.3. Additional Equipment and Reagent required

### Standard laboratory equipment

- 96 well tissue-culture grade, flat-bottomed microplates
- $\text{CO}_2$  incubator
- Centrifuge
- ELISA reader

### For the determination of the activity of recombinant, human TGF- $\beta$ 1 on sensitive cells

- Culture medium, such as MEM (Earle) containing 10% FCS (fetal calf serum), heat-inactivated, and 2 mM L-glutamine.
- TGF- $\beta$ 1 stock solution: 1  $\mu$ g/ml TGF- $\beta$ 1, human, recombinant in filtered 1 mg/ml PBS and BSA.
- Cell Proliferation ELISA, BrdU (colorimetric)\*

*i* Saturate all materials that come into contact with TGF- $\beta$ 1 in HCl/BSA solution (5 mM HCl, 0.1% BSA), such as microplates, filters, and pipette tips.

## 1.4. Application

*i* TGF- $\beta$ 1 may only be used in life science research studies.

The TGF- $\beta$  peptides affect many functions in nearly all cells (multifunctional growth factor). The nature of their actions on a particular target cell is critically dependent on many parameters including the cell type and its state of differentiation, the growth conditions, and on other growth factors present. Target tissues (cells) of TGF- $\beta$  include mesenchyme (fibroblasts), muscle (skeletal, cardiac, and smooth muscle cells), bone (osteoblasts), hematopoietic (bone marrow cells), and immune system (B and T cells, monocytes, and macrophages), endothelium (endothelial cells), and epithelia (hepatocytes, keratinocytes).

## 2. How to Use this Product

### 2.1. Before you Begin

#### General Considerations

##### Primary structure

Two identical polypeptide chains (each with 112 amino acids, connected by disulfide bridges) are identical to that of natural, human TGF- $\beta$ 1.

**i** *The primary structure of recombinant, human TGF- $\beta$ 1 is identical to that of natural, human TGF- $\beta$ 1.*

#### Working Solution

Dilute the concentrated TGF- $\beta$ 1 solution (1  $\mu$ g/ml) with PBS or culture medium containing 1 mg/ml (0.1%) BSA or HSA (human serum albumin), or 1 to 10% serum.

### 2.2. Protocols

#### Determination of the activity of recombinant, human TGF- $\beta$ 1 on sensitive cells

The following steps were performed using Mv1Lu cells.

- 1 Seed sensitive cells, such as Mv1Lu cells (mink lung epithelial cell line, ATCC CCL64) at a concentration of  $1.6 \times 10^5$  cell/ml in culture medium into the wells of a 96-well microplate (50  $\mu$ l/well).

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- 2 Add various amounts of TGF- $\beta$ 1 (final concentration approximately 0.001 ng/ml to 25 ng/ml) diluted in culture medium into the wells of the microplate (50  $\mu$ l/well), see section, **Results, Figure 1**.

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- 3 Incubate the cells for 18 hours at +37°C and 6.5% CO<sub>2</sub>.

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- 4 Perform the Cell Proliferation ELISA, BrdU (colorimetric)\* as described in the Instructions for Use using a 6 hour incubation time with BrdU.

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## 2.3. Parameters

### Biological Activity

Biological activity is  $\leq 0.1$  ng/ml, at least the same specific activity ( $EC_{50}$ ) compared to the indicated standard is guaranteed.

**i** *The biological activity of this product may vary in different in vitro applications. Determine the optimal concentration range for specific applications.*

### Molecular Weight

25,000 Da

### Purity

$\geq 98$  % pure as determined by SDS-PAGE.

Endotoxin level:  $\leq 10$  EU/ml (LAL).

**i** *1 EU corresponds to 0.1 ng.*

### Specificity

Human TGF- $\beta$ 1 is active on mouse, rat and human cells.

### Unit Definition

#### $EC_{50}$ definition

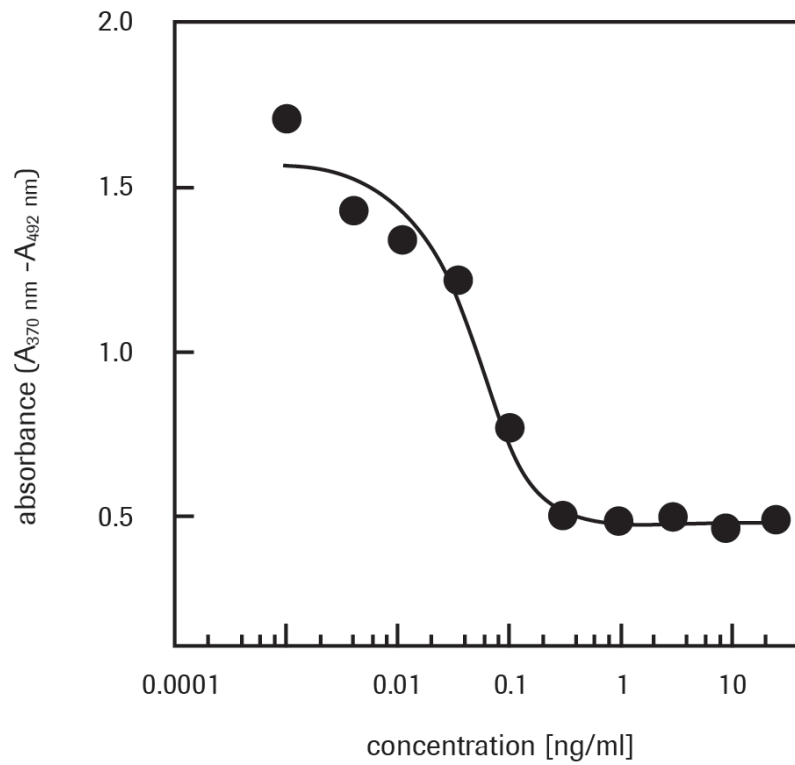
The amount of hTGF- $\beta$  that is required to mediate half-maximal inhibition of DNA synthesis (BrdU incorporation) with Mv1Lu cells.

### Working Concentration

Recombinant, human TGF- $\beta$ 1 exerts its biological activity in the concentration range of 0.01 to 10 ng/ml.

### 3. Results

#### Inhibition of BrdU incorporation into Mv1Lu cells



**Fig. 1:** Inhibition of BrdU incorporation into Mv1Lu cells in response to recombinant, human TGF-β1.

## 4. Additional Information on this Product

### 4.1. Test Principle

The TGF- $\beta$ -related family of polypeptides includes a group of closely homologous dimeric proteins. The three distinct molecular forms of TGF- $\beta$  which have been identified in mammals have been designated TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3. The three closely related TGF- $\beta$  molecules are first synthesized as larger precursor polypeptides that are then processed to yield 12.5 kD monomers. The biologically active, mature 25 kD polypeptide consist of two identical disulfide-linked monomers. In the mature sequences, human TGF- $\beta$ 2 and TGF- $\beta$ 3 genes are approximately 80% homologous to TGF- $\beta$ 1, while TGF- $\beta$ 3 is 72% homologous to TGF- $\beta$ 2.

### Preparation

Recombinant, human TGF- $\beta$ 1 is produced in CHO cells and purified by standard chromatographic techniques.





### 4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

## 5. Supplementary Information

### 5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
 <i>Information Note: Additional information about the current topic or procedure.</i>	
 <b>Important Note: Information critical to the success of the current procedure or use of the product.</b>	
 etc.	Stages in a process that usually occur in the order listed.
 etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

### 5.2. Changes to previous version

Layout changes.  
Editorial changes.

### 5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Cell Proliferation ELISA, BrdU (colorimetric)	1 kit, 1,000 tests	11 647 229 001



## 5.4. Trademarks

All product names and trademarks are the property of their respective owners.

## 5.5. License Disclaimer

For patent license limitations for individual products please refer to:

**List of biochemical reagent products.**

## 5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

## 5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

## 5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

