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# **$\beta$ -1,4-Galactosyltransferase, rec. EQ human, expressed in CHO-K1 cells**

 **Version: 01**

Content Version: June 2021

Solution

<b>Cat. No. 08 098 182 103</b>	0.4 g
<b>Cat. No. 08 098 182 101</b>	1.5 mg

**Store the product at –15 to –25°C.**

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# 1. General Information

## 1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	$\beta$ -1,4-Galactosyltransferase, rec.	$\beta$ -1,4-Galactosyltransferase, rec. in enzyme storage buffer: 50 mM Tris-HCl, 100 mM NaCl, pH 8.4 at +20°C.	08 098 182 101	1 vial, 1.5 mg
			08 098 182 103	1 vial, 0.4 g

## 1.2. Storage and Stability

### Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	$\beta$ -1,4-Galactosyltransferase, rec.	Store at $-15$ to $-25^{\circ}\text{C}$ .

## 1.3. Additional Equipment and Reagent required

### For galactosylation of target protein

 See section, **Working Solution** for information on preparing Reaction buffer.

- Reaction buffer
- UDP-Galactose\*, 10 mM
- $\text{MnCl}_2$ , 20 mM
- MES, 100 mM
- IgG, 1 mg

## 1.4. Application

For *in vitro* galactosylation of all GlcNAc1-2Man units on glycoproteins and complex molecules such as monoclonal antibodies (MAB), use  $\beta$ -1,4-Galactosyltransferase (B4GalT1) and UDP-Galactose (UDP-Gal)\*.

## 2. How to Use this Product

### 2.1. Before you Begin

#### General Considerations

##### Assay conditions

Conditions may vary depending on target molecules and applications, such as incubation time, enzyme concentration, etc. If you observe precipitation, reduce the  $\text{MnCl}_2$  concentration stepwise until 5 mM.

**⚠ Do not combine the reaction buffer containing  $\text{MnCl}_2$  with a phosphate buffer.**

**i** For further information on, for example, galactosyltransferase activity or assay conditions for other targets, contact your CustomBiotech representative.

##### Analytics

Samples were analyzed by electrospray ionization mass spectrometry and the content of G2+0Ga, G2+1Ga, and G2+2Ga N-glycans was determined.

- Instrument: Synapt G2 HDMS device (Waters, UK)
- Software: MassLynx V 4.1

#### Working Solution

##### Preparation of reaction buffer

Prepare the Reaction buffer containing 10 mM UDP-Gal, 20 mM Manganese(II) chloride ( $\text{MnCl}_2$ ), and 100 mM MES, pH 6.5:

- 1 Dissolve 61 mg UDP-Gal, 25.2 mg  $\text{MnCl}_2$ , and 195 mg MES in 6 ml water.
- 2 Adjust pH to 6.5.
- 3 Adjust to a final volume of 10 ml with water.

## 2.2. Protocols

### Galactosylation of target protein

The optimal reaction conditions for galactosylation of a target protein can be different for each application. Use the following protocol as a starting point. Optimize reaction conditions, for example, with different enzyme-IgG ratios to determine the optimal conditions for each application.

**i** See section, **Working Solution** for information on preparing the Reaction buffer.

- 1 Set up the galactosylation reaction, containing 976 µg UDP-Gal, 30 µg B4GalT1, and 1 mg IgG.  
– Into a reaction tube, pipette 160 µl Reaction buffer, 33.3 µl IgG (30 mg/ml, pH 6), 5.5 µl β-1,4-Galactosyltransferase, and 1.2 µl water.

- 
- 2 Incubate the reaction at +37°C.

- 
- 3 Stop the reaction by placing the reaction tube at –15 to –25°C.

- 
- 4 Analyze the samples.

**i** To plot the kinetics for each application, set up several identical reactions and stop them at different time points, for example, 2, 8, or 24 hours.

## 2.3. Parameters

### EC-Number

EC 2.4.1.38 (β-N-acetylglucosaminylglycopeptide β-1,4-galactosyltransferase)

### Molecular Weight

39.5 kDa, determined by cDNA.

## 3. Results

As an example, nearly 100% degalactosylated but highly N-acetyl-glucosaminylated monoclonal antibodies IgG1 and IgG4 are used for kinetics.

### Kinetics of $\beta$ -1,4-Galactosyltransferase with IgG1

90% biantennary galactosylation of IgG1 is observed after 8 hours (G2). After 24 hours, IgG1 is fully galactosylated. No intrinsic galactosidase activity is observed at longer incubation times.

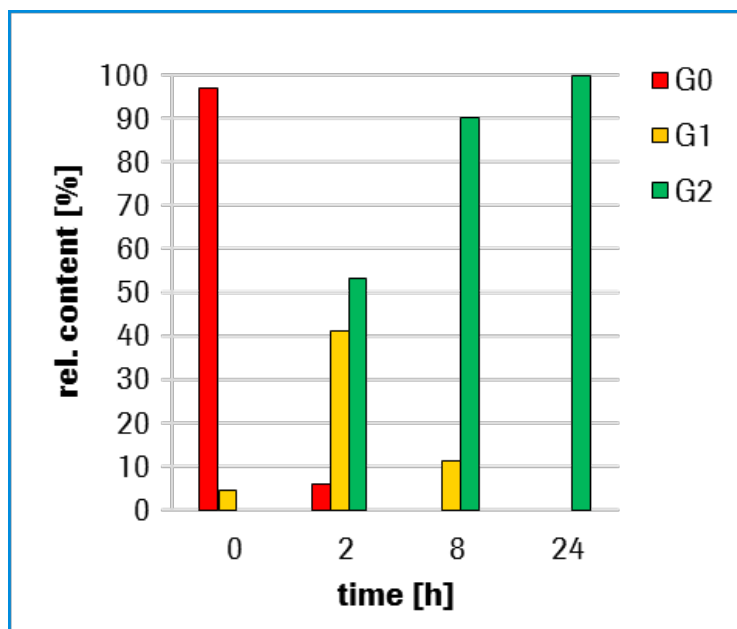


Fig. 1: Time course of galactosylation of IgG1 using recombinant B4GalT1.

### Kinetics of $\beta$ -1,4-Galactosyltransferase with IgG4

After 2 hours, more than 70% biantennary galactosylation of IgG4 is observed (G2). After 8 hours, IgG4 is fully galactosylated. No intrinsic galactosidase activity is observed at longer incubation times.

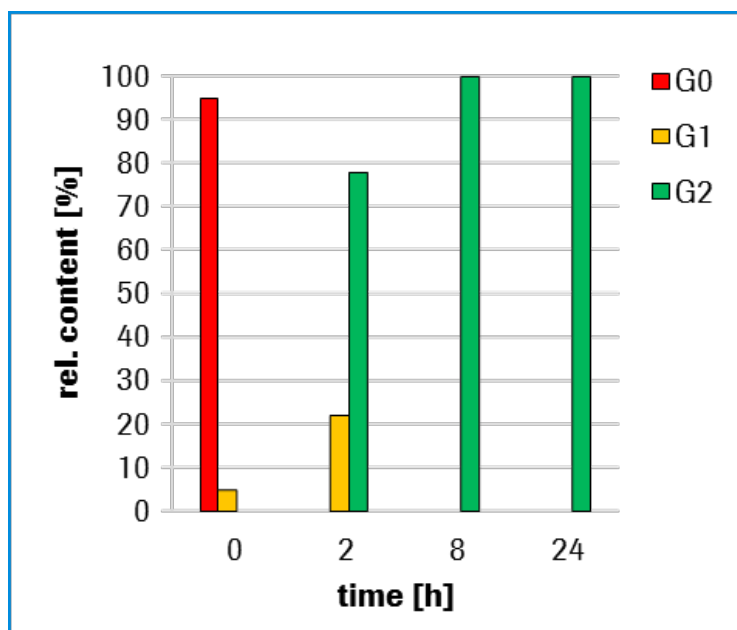


Fig. 2: Time course of galactosylation of IgG4 using recombinant B4GalT1.

## 4. Supplementary Information

### 4.1. 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

 **Information Note:** Additional information about the current topic or procedure.

 **Important Note:** Information critical to the success of the current procedure or use of the product.

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

\* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

### 4.2. Changes to previous version

First version.

### 4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
UDP-Galactose animal-free	custom fill	07 703 562 103

## 4. Supplementary Information

### 4.4. Trademarks

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

### 4.5. License Disclaimer

For patent license limitations for individual products please refer to:

<http://documentation.roche.com>.

### 4.6. Regulatory Disclaimer

For further processing only.

### 4.7. Safety Data Sheet

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

### 4.8. Contact and Support

For additional documentation such as certificates and safety data sheets, please visit [documentation.roche.com](http://documentation.roche.com).

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