**α1-3, 6 Galactosidase**

**Description:** α1-3, 6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3, 6 linked D-galactopyranosyl residues from oligosaccharides.

**Specificity:**
- Gal α 1→3 R
- Gal α 1→6 R

**Detailed Specificity:** Specificity can vary depending on incubation time and branching structure.

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-α-galactose from 1 nmol Galα1-3Galβ1-4Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAcβ1-6Fucα1-2 in 1 hour at 37°C in a total reaction volume of 10 µl.

**Source:** Cloned from *Xanthomonas manihotis* and expressed in *E. coli* (1).

**Supplied in:** 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM Na₂EDTA.

**Reagents Supplied with Enzyme:**
- 10X G6 Reaction Buffer
- 100X BSA

**Reaction Conditions:**
- 1X G6 Reaction Buffer
- 50 mM Sodium acetate (pH 5.5 @ 25°C) and 5 mM CaCl₂, Supplement with 100 µg/ml BSA. Incubate at 37°C.

**Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.**

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-α-galactose from 1 nmol Galα1-3Galβ1-4Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAcβ1-6Fucα1-2 in 1 hour at 37°C in a total reaction volume of 10 µl.

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**Figure 1:**

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**A) 0.1 nm/ml substrate, 1 hour incubation**

Galα(1→6)Glcα(1→2)Fru

**B) 0.1 nm/ml substrate, 18 hour incubation**

Galα(1→3)Galβ(1→4)GlcNAcβ(1→4)

NeuAc(2→6)Galβ(1→4)GlcNAcβ(1→2)Manα(1→3)

Galβ(1→4)GlcNAcβ(1→2)Manα(1→6)

Galβ(1→4)GlcNAcβ(1→2)Manα(1→6)

Manβ(1→4)GlcNAcβ(1→4)GlcNAcβ(1→6)Asn

Galα(1→3)Galβ(1→4)GlcNAcβ(1→6)

Fucα(1→6)

**C) 0.1 nm/ml substrate, 1 hour incubation, not cleaved**

Galα(1→3)Galα(1→3)Glc

Fucα(1→2) Fucα(1→2)

(See other side)
### Glycosidase Assays:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>α-N-Acetylgalactosaminidase</strong></td>
<td>GalNAcc1-3(Fucx1-2)Galβ1-4Glc-AMC</td>
<td>ND</td>
</tr>
<tr>
<td><strong>α-Fucosidase</strong></td>
<td>Fucx1-2Galβ1-4Glc-AMC</td>
<td>ND</td>
</tr>
<tr>
<td><strong>β-Galactosidase</strong></td>
<td>Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC</td>
<td>ND</td>
</tr>
<tr>
<td><strong>α-Neuraminidase</strong></td>
<td>Neu5Acx2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC</td>
<td>ND</td>
</tr>
</tbody>
</table>

### Unit Definition Assay:

Two fold serial dilutions of α1-3, 6 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G6 Reaction Buffer and 1X BSA in a 10 µl reaction. The reaction mix is incubated for 1 hour at 37°C. Separation of reaction products is visualized via thin layer chromatography (2).

**Specific Activity:** 137,000 units/mg

**Molecular Weight:** 70,000 daltons.

**Quality Assurance:** No contaminating exoglycosidase or proteolytic activity could be detected.

<table>
<thead>
<tr>
<th>Quality Controls</th>
<th>Assay Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glycosidase Assays:</strong></td>
<td>12 units of α1-3, 6 Galactosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products are analyzed by TLC for digestion of substrate.</td>
</tr>
</tbody>
</table>

### Physical Purity:

Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

No other glycosidase activities were detected (ND) with the following substrates:

- **α-N-Acetylgalactosaminidase:** GalNAcc1-3(Fucx1-2)Galβ1-4Glc-AMC
- **α-Fucosidase:** Fucx1-2Galβ1-4Glc-AMC
- **β-Galactosidase:** Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC
- **α-Neuraminidase:** Neu5Acx2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC

### Protease Assay:

After incubation of 28 units of α1-3, 6 Galactosidase with 0.2 nmol of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

**Note:** Recommended storage temperature is 4°C. Avoid repeated freeze/thaw cycles.

### Heat Inactivation:

65°C for 10 minutes.

**References:**

U.S. Patent No. 5,770,405