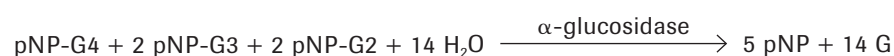
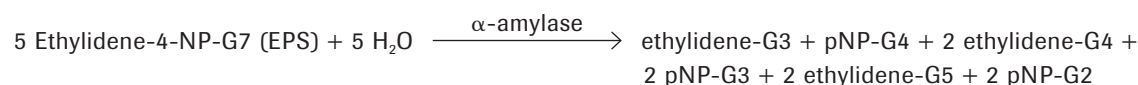


## Pancreatic $\alpha$ -Amylase

### Test principle: Enzymatic colorimetric



A variety of methods have been described for determining pancreatic  $\alpha$ -amylase. These include radio- and enzyme-immunoassays, the partial inhibition of salivary  $\alpha$ -amylase by an inhibitor derived from wheat germ, and calculation of pancreatic  $\alpha$ -amylase from remaining and total amylase activities. The kinetic method described here is based on inhibition of the activity of human salivary  $\alpha$ -amylase by two different monoclonal antibodies, and the well-proven cleavage of 4,6-ethylidene-(G7)-1,4-nitrophenyl-(G1)- $\alpha$ ,D-maltoheptaoside (Ethylidene Protected Substrate, or EPS) by pancreatic  $\alpha$ -amylase, followed by hydrolysis of all the degradation products to p-nitrophenol with the aid of

$\alpha$ -glucosidase (100% chromophore liberation). The results of this method correlate with those obtained by HPLC.

In the first incubation step, the activity of human salivary  $\alpha$ -amylase is inhibited by two different monoclonal antibodies (without affecting the pancreatic  $\alpha$ -amylase). In the second reaction step, defined oligosaccharides, such as 4,6-ethylidene-(G7) p-nitrophenyl-(G1)- $\alpha$ ,D-maltoheptaoside (Ethylidene-4-NP-G7) are cleaved under the catalytic action of pancreatic  $\alpha$ -amylases. The G2PNP, G3PNP, and G4PNP fragments formed are completely hydrolyzed to p-nitrophenol and glucose by  $\alpha$ -glucosidase.

### Proposed reagent composition approximately 5+1 formulation

#### Reagent 1

| Composition  | Concentration | Catalog Number                   |
|--|---------------|----------------------------------|
| Buffer (HEPES, pH 7.0)   | 52.4 mmol/l   | 10 172 944 103                   |
| NaCl   | 87 mmol/l     |                                  |
| MgCl <sub>2</sub>  | 12.6 mmol/l   |                                  |
| CaCl <sub>2</sub>  | 0.075 mmol/l  |                                  |
| $\alpha$ -Glucosidase  | >4 kU/l       | 11 626 329 103                   |
| Monoclonal antibodies against salivary $\alpha$ -amylase (mouse) | 97 mg/l       | 11 543 598 103<br>11 543 601 103 |
| Detergent, preservative, such as Polidocanol MIT                 |               | 10 831 620 103<br>11 085 905 103 |

#### Reagent 2

| Composition                                      | Concentration | Catalog Number                   |
|--|---------------|----------------------------------|
| Buffer (HEPES, pH 7.0)                           | 52.4 mmol/l   | 10 172 944 103                   |
| Ethylidene-4-NP-G7 (EPS)                         | 22 mmol/l     | 10 880 078 103                   |
| Detergent, preservative, such as Polidocanol MIT |               | 10 831 620 103<br>11 085 905 103 |

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