

# Genaxxon BioScience

## β-Agarase

### Manual

#### Agarose digestion

1. Determine the weight of a microcentrifuge tube first.
2. Cut out the desired band from the agarose gel as precise as possible. Determine the weight of the agarose slice after transfer to the 1.5 ml microcentrifuge tube and melt the agarose at 70°C completely.  
**Attention:** Incubation at elevated temperatures may denature the DNA. Digest no more than 200-500 mg agarose per tube. Cut gel slices larger than 200 mg into smaller pieces. If the agarose is completely melted, the hydrolysis of the agarose will be incomplete, too.
3. Transfer the tube to a water bath adjusted to 40-42°C for approximately 5 minutes to equilibrate to the optimum reaction temperature prior to adding agarase.
4. Add one unit of β-agarase per 100 µl (appr. 100 mg) of 1% molten agarose and incubate for 30 minutes at 40-42°C. When using higher percentage agarose, the amount of agarase should be proportionally increased.

#### Purification of large DNA fragments

1. DNA fragments larger than 30 kb must be handled very carefully to prevent mechanical shearing. Therefore, centrifuge at 15000xg for 10 minutes to pellet undigested carbohydrates.
2. Remove oligosaccharides and agarase by dialysis or carry out subsequent manipulations with DNA in the digested agarose solution.

#### Purification of small DNA fragments

1. DNA fragments smaller than 30 kb can be isolated from the digested agarose by ethanol precipitation. Add salt to the hydrolysed agarose: Either ammonium acetate (final concentration 2.5 M) or sodium acetate (final concentration 0.3 M).  
**Attention:** To prevent co-precipitation of agarose-oligosaccharides, perform the precipitation at room temperature with ammonium acetate rather than sodium acetate. **NOTE:** T4 polynucleotide kinase is inhibited by ammonium ions at concentrations above 7 mM. Use sodium acetate, if the 5'-ends of the isolated DNA will be labelled with T4 Kinase.
2. Chill on ice for 5 minutes, centrifuge at 15000xg for 10 minutes to pellet undigested carbohydrates.
3. Transfer the supernatant to a clean new tube and either add 1 volume of isopropanol or 2-3 volumes of ethanol. Mix gently and incubate for at least 30 minutes at 0°C to 22°C.  
**Attention:** If the DNA fragments are smaller than 500 bp or if the DNA concentration is lower than 0.05 µg/mL, incubate overnight at 0°C to 22°C.
4. Centrifuge at 15000xg for 15 minutes. Remove the supernatant and dry pellet. Resuspend in an appropriate buffer for subsequent manipulation.

The following agaroses from Genaxxon are suited for digestion by β-agarase: M3046 GenAgarose Tiny; M3048 GenAgarose LMH; M3045 GenAgarose Plus.

#### β-agarase activity in different buffer systems

150%	50 mM Bis-Tris, pH 6.5, 1 mM EDTA	100 mM Bis-Tris, pH 6.5, 10 mM EDTA	10 mM Tris, pH 7.6, 5 mM EDTA, pH 8.0, 0.1 M NaCl
120%	90 mM Tris-Phosphate (TBE-buffer), 2 mM EDTA	40 mM Tris-Acetate (TAE- buffer), 1 mM EDTA	
100%	45 mM Tris-Borate (TBE- buffer), 1 mM EDTA		