



## CALCOFLUOR WHITE : PREPARATION (Lot 40301a)

**C-CLFR10**

**07/11**

### **Availability:**

Calcofluor White (Tinopal 5BM) is supplied as a powder in a brown glass bottle. This bottle should be stored dry and dark at room temperature.

### **Preparation of reagents:**

#### **A. Hydrochloric acid (1 M)**

Dilute 82.3 mL of 37% (w/w) HCl with distilled water in a 1 litre volumetric flask and adjust to the mark.

#### **B. Tris/HCl buffer solution (0.1M, pH 9.0 ± 0.2)**

Dissolve 12.1g tris (hydroxymethyl) aminomethane ( $C_4H_{11}NO_3$ ) (Sigma cat. no. T 1503) in 800 mL of distilled water. Add 40 mL of 1 M HCl and adjust the pH to 9.0 with additional 1 M HCl. Transfer the solution to a 1 litre volumetric flask and adjust to the mark with distilled water.

Filter the buffer solution through a Whatman GF/A glass fibre filter paper and finally degas the buffer under vacuum.

#### **C. Calcofluor stock solution**

Add 350 mg of Calcofluor White ( $C_{40}H_{44}N_{12}O_{10}S_2$ ) to a 100 mL volumetric flask. Add 70 mL of distilled water and a few drops of sodium hydroxide (increases the pH to 10-11). Dissolve the Calcofluor. Add 10 mL of Triton X-100 solution and adjust to volume with distilled water.

Store this solution in a light-proof bottle (preferably covered with Al-foil) at 4-10°C.

#### **D. Calcofluor working reagent**

Transfer 10 mL of Calcofluor stock solution (B) to a 1 litre volumetric flask and adjust to volume with Tris/HCl buffer solution. Store this solution in a light-proof bottle.

**Note:** This solution should be prepared fresh daily and used the same day.

This solution can be used, in conjunction with CFA Beta-Glucan Standard (Megazyme cat. no. P-BGCFA) (formerly P-FIAGS) or Barley Beta-Glucan (Medium Viscosity; Megazyme cat. no. P-BGBM), for the measurement of beta-glucan in wort and beer.