DESCRIPTION AND INSTRUCTION FOR USE

PRODUCT	**A+ Starch hydrolized** Acid hydrolized starch and agarose premix for protein horizontal zone electrophoresis
Item-Nr.	#1350

Properties
White powder which, when processed according to the procedure given below, forms translucent gels which can be cut into thin slabs and will have properties equivalent to material originally supplied by Connought Laboratories in Toronto.
The Smithies formulations have been developed for plasma proteins, the Leineman method for isozyme analysis in plant tissue. A compilation of recipies for the separation of many other proteins can be found at Britton-Davidian, Janice, Starch Gel Electrophoresis in Vertebrates (1993) Methods in Enzymology 224, 98-112.

Preparation of gels – Smithies standard method Smithies, O., (1955) Biochem. J. **61**, 629-633.
For best results please follow closely the proposed procedure (250 ml)

1. **Water bath:** Prepare a bath of slightly boiling water on a magnetic stirrer equipped with hot plate.
2. **Starch suspension:** Suspend 25 g starch in 240 ml cold buffer solution contained in a 500 ml vacuum suction flask. Be careful to disperse the starch evenly without lumps.
3. **Stirring magnet:** Use a large size rod suitable for efficient mixing of very viscous media.
4. **Heating:** Close the mouth of the vacuum flask by means of aluminium foil or Parafilm in order to prevent evaporation. Insert the flask into the water bath, fix with a clamp and stir gently. As a temperature of 55 °C is reached, the gel becomes very viscous. Stir efficiently to keep the gel homogenous, assisted by occasional shaking or stirring with a glass rod. Ca 20 minutes after inserting the flask into the water bath, the viscosity has decreased significantly. Continue stirring for additional 5 minutes at slower speed in order to expel air bubbles.
5. **Deaeration** To the hot solution in the vacuum flask apply vacuum aspirator, boil ca 15 seconds.
6. **Pouring of the gel:** Pour the hot gel into the mold and cover it. Store at least 2 hours at 4 °C

Preparation of buffers according to Smithies
For horizontal gels – 0.023 M boric acid - 0.0092 M NaOH. pH 8.6.
1 Liter is prepared from 1.42 g boric acid + 0.368 g NaOH or 9.2 ml 1N sodium hydroxide solution
For vertical gels - 0.026 M boric acid – 0.0104 M NaOH, pH 8.6.
1 Liter is prepared from 1.61 g boric acid + 0.416 g NaOH or 10,4 ml 1N sodium hydroxide solution

Preparation of gels – Leinemann microwave method Institut für Forstgenetik und Forstpflanzenzüchtung, Universität Göttingen. Please follow closely the proposed procedure (250ml)

1. **Hot buffer:** In a 500 ml vacuum suction flask place 160 ml TRIS citrate buffer, heat to boiling in a microwave oven.
2. **Starch suspension:** Suspend 28 g in 80 ml TRIS-citrate buffer in a baker, add 5 g sucrose and shake well. Transfer the suspension into the hot TRIS citrate buffer in the suction flask.
3. **Microwave heating:** Place the suction flask back into the microwave oven and heat the suspension to boiling for 1 minute. Interrupt for stirring or shaking.
4. **De-aeration:** To the hot suspension in the vacuum flask apply vacuum aspirator.
5. **Pouring of the gel:** Pour the hot gel into the mold. Leave the gel for at least 30 minutes at room temperature.

Preparation of TRIS citrate buffer according to Leinemann:
0.023 M citric acid – 0.05 M TRIS
1 Liter is prepared from 2.75 g citric acid monohydrate + 6.07 g Tris hydroxylaminomethane