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Acrylamide/Bisacrylamide 30% (19:1)

GB16.3019 – 500 ml

(FOR RESEARCH ONLY)

Product Description

Ready-to-Use Acrylamide-Bisacrylamide solutions are aqueous solutions of ultrapure, 3x re-crystallized acrylamide and molecular biology grade bisacrylamide, prepared with ultrapure water, 0.2 µm filtered, and suitable for electrophoresis of proteins and nucleic acids. The concentration of this product (30%) is determined by the total (T) weight of both acrylamide and bisacrylamide (T= 30g per 100ml), in which the mix ratio is 19:1 resulting in a cross-linking (C) of 5%.

Principle

The range of protein sizes to be separated is determined by the pore size of a polyacrylamide gel. Control of the pore size is accomplished by regulating both T and C. The relationship between T and pore size is nearly linear; Gels with a higher percentage of T have smaller pores, and are used to separate smaller proteins. However, the relationship between C and pore size is more complex with a minimum occurring at about 5% bisacrylamide. A ratio between acrylamide and bisacrylamide of 19:1 (5% C) is suitable for the separation of small peptides, whereas a ratio of 29:1 [this product] is commonly used for the separation of "normal sized" proteins. High molecular weight proteins are best separated using a 37,5:1 mix ratio.

Caution

Ready-to-Use Acrylamide-Bisacrylamide solutions eliminate the hazard of handling powdered acrylamide, however, good laboratory practices are to be used. Always work in a well ventilated area (e.g. a fume hood) and wear protective clothing and goggles. Acrylamide is a neurotoxin and may cause cancer or genetic defects. Suspected of damaging fertility of the unborn child. Prolonged or repeated exposure may cause organ damage. Harmful in contact with skin and if swallowed. Causes skin and serious eye irritation. After contact with skin or eyes, wash immediately with plenty of water. If you feel unwell, seek medical advice (show label where possible). Empty containers must be disposed of as hazardous waste.

Components and Storage

Acrylamide-Bisacrylamide solutions are stable for several months at room temperature, however, if stored at 2-8°C, the solutions are stable for at least one year.

PROTOCOL

The volume of Acrylamide/Bisacrylamide stock solution required in order to obtain the desired gel concentration (<30%) can be calculated as follows:

$$V = c \times \frac{V_{tot}}{30\%}$$

Where V is the required volume of the 30% stock solution, c is the desired concentration and V_{tot} the total desired volume. In the table below, some values are presented for the most commonly used percentages in the Laemmli system¹.

Electrophoresis systems vary in sizes and comb thickness and one can often run more than one gel at the time. The amounts of the components presented in the table are based on a 10ml polyacrylamide gel and depending the total volume desired may be adjusted proportionally. Note that the volume of the stacking gel already has been adjusted to a final volume of 4ml.

TEMED and 10% APS should only be added immediately prior to pouring the gel. Before pouring, swift gently to homogenize polymerization.

Percentage	4% (stacking gel)	7,5%	10%	12%	15%	20%
acrylamide/bisacrylamide solution 30% (ml)	0,53	2,50	3,33	4,00	5,00	6,67
0.5M Tris-HCl pH 6.8 (ml)	1,00	-	-	-	-	-
1,5M Tris-HCl pH 8.8 (ml)	-	2,50	2,50	2,50	2,50	2,50
ddH ₂ O (ml)	2,39	4,84	4,00	3,33	2,33	0,66
10% SDS (μl)	40	100	100	100	100	100
TEMED (μl)	4	5	5	5	5	5
10% APS (μl)	20	50	50	50	50	50
Total Volume (ml)	4	10	10	10	10	10

REFERENCES

1. Laemmli, UK (1970) *Nature* **227**: 680-685

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