3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Ammonium Sulfate Solution, 4.1 M
Saturated at 25 °C

Product Number **A 5479**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

Ammonium sulfate is widely used for precipitation and fractionation of proteins[1] as well as for the crystallization of proteins [2,3,4] and protein-nucleic acid complexes.[5] Ammonium sulfate is also utilized in hydrophobic interaction chromatography[6,7,8] and antibody purification.[9,10]

Ammonium sulfate acts by pulling water molecules away from the non-polar units of proteins. The decrease in available water molecules increases the surface tension and enhances hydrophobic interactions, thus allowing the protein to precipitate from a solution or bind to a hydrophobic column. The use of ammonium sulfate confers the following advantages:

1) High concentrations of ammonium sulfate inhibit microbial growth and maintain the protein in a folded state.
2) The low density of saturated solutions (1.25 g/cm^3) allows pelleting of proteins by centrifugation.
3) A low heat of solubilization avoids the risk of protein denaturation that can occur when the sample temperature increases.

The saturation concentration of an ammonium sulfate solution is temperature dependent. Lower temperatures will decrease the concentration at which the solution is saturated. A saturated solution of ammonium sulfate is 4.1 M at 25 °C, but is 3.8 M at 0 °C, the difference in molarity being 7%. Accordingly, a saturation table based on the addition of crystalline ammonium sulfate varies with temperature. For this reason, an aliquot from a saturated solution at 25 °C may be stored at the temperature desired for the application. For the original concentration to be maintained with accuracy, the saturated solution at 25 °C should contain no insoluble ammonium sulfate crystals when aliquoted.

The product is tested and found free of DNAse, RNAse, and protease activities.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The reagent is supplied as a ready-to-use, clear, colorless liquid. If crystals are observed in the bottle, this may be due to the temperature of the solution falling below 25 °C during shipping or storage. Warm the solution to 30–37 °C and gently mix to redissolve the crystals. Allow the solution to cool to room temperature prior to use.

If protein precipitation or hydrophobic chromatography is to be carried out at a temperature below room temperature [e.g. 0 °C (ice) or 2 – 8 °C (cooler)], remove an aliquot of the product and cool it to the temperature that will be used. Ammonium sulfate crystals will appear and settle at the bottom of the bottle. Be careful not to disturb the crystals while removing the saturated ammonium sulfate supernatant.

Storage/Stability

The product is stable for at least two years when stored at room temperature.

Procedure

Protein Precipitation

For protein purification, often two precipitation steps are carried out on a given protein sample. The first step is performed at an ammonium sulfate concentration below that required to precipitate the protein(s) of interest. Accordingly the protein(s) of interest remain in the supernatant, while other proteins precipitate and are collected in the pellet upon centrifugation. The second step is performed at an ammonium sulfate concentration high enough to precipitate or pellet the protein(s) of interest. Additional proteins may remain in the supernatant.

The procedure presented here is to be used as a guideline only. Two ammonium sulfate precipitation steps are performed, involving the addition of the saturated ammonium sulfate solution to raise the percent saturation of the sample solution initially from 0% to 35% and then in the second step, from 35% to 70%. **The appropriate number of precipitation steps and the concentration (percent saturation) of ammonium sulfate required will vary depending on the protein(s) of interest and must be determined empirically.**

1. Prepare a protein sample at a concentration of at least 1 mg/ml in a buffered solution (≥50 mM buffer). Cool the protein sample and the saturated ammonium sulfate solution (Product Code A 5479) to the desired working temperature. The ammonium sulfate solution typically has a pH in the range of 5 to 6, so a slight pH shift may occur if the protein sample is not adequately buffered.

2. Determine the volume of the saturated ammonium sulfate solution required to give the desired ammonium sulfate concentration (percent saturation) in the protein sample using Table 1 (Appendix).

   For this example the protein sample has a starting volume of 100 ml and an ammonium sulfate concentration of 0%. The first precipitation step will be performed at 35% saturation with ammonium sulfate. Using the left side of Table 1, locate the row corresponding to initial ammonium sulfate concentration (0%). Follow this row across the table to the column corresponding to the final ammonium sulfate concentration (35% saturation). The multiplication factor corresponding to raising the ammonium sulfate concentration from 0 to 35% saturation is 0.538.

   Multiply the initial sample volume (100 ml) by the multiplication factor (0.538) to calculate the volume of the saturated ammonium sulfate solution required (53.8 ml).

3. Slowly add the calculated volume (53.8 ml) of the saturated ammonium sulfate solution to 100 ml of the protein sample with gentle stirring.

4. Allow the solution to incubate at the desired temperature for a minimum of 20 minutes. Some proteins may require a longer period of time to precipitate, so the optimal length of time for precipitation should be determined empirically for each sample.

5. At the desired temperature, centrifuge the sample at a minimum of 1,000 x *g* for at least 5 minutes. Repeat the centrifugation step as needed to ensure the complete precipitation of protein. Carefully decant or pipette the supernatant from the pellet.

6. For this example the protein of interest remains in the supernatant from step 5 (35% saturation) and a second precipitation step at 70% saturation is required to pellet the protein of interest. Repeat the calculations detailed in step 2 to determine the volume of saturated ammonium sulfate solution required to bring the ammonium sulfate concentration to 70% saturation.

   Locate the row corresponding to ammonium sulfate concentration, now 35% saturation, from Table 1 and move across the table to the column corresponding to the desired final ammonium sulfate concentration (70% saturation). The multiplication factor corresponding to raising the ammonium sulfate concentration from 35 to 70% saturation is 1.167.

   Multiply the current sample volume (153.8 ml) by the multiplication factor (1.167) to calculate the volume of the saturated ammonium sulfate solution required (179.5 ml).

7. Slowly add the calculated volume (179.5 ml) of the saturated ammonium sulfate solution to the 153.8 ml of protein sample (supernatant from step 5) with gentle stirring.

8. Allow the solution to incubate at the desired temperature for a minimum of 20 minutes. Some proteins may require a longer period of time to precipitate, so the optimal time for precipitation should be determined empirically for each sample.

9. At the desired temperature, centrifuge the sample at a minimum of 1,000 x *g* for at least 5 minutes. Repeat the centrifugation step as needed to ensure the complete precipitation of protein. Carefully decant or pipette the supernatant from the pellet.

10. Dissolve the protein pellet in the desired buffer and store at the appropriate temperature.

Hydrophobic Chromatography

Hydrophobic chromatography is typically performed by applying the protein sample with a high salt concentration (e.g. 1 – 2 M ammonium sulfate) to a column for binding. The protein sample buffer and the column equilibration buffer should have the same ammonium sulfate concentration, typically just below the concentration required to precipitate the protein(s). The proteins are then eluted from the column with a lower ammonium sulfate concentration, typically using a gradient from high to low concentrations of ammonium sulfate. Often the low concentration solution is simply the buffer without ammonium sulfate.

1. Remove an aliquot of the saturated ammonium sulfate solution and adjust to the desired working temperature.

2. Dilute the saturated ammonium sulfate solution with water and/or a concentrated buffer solution to the desired concentration for column equilibration and gradient.

   Calculation:

   Volume of saturated ammonium sulfate solution required =

$$\frac{\text{Desired final volume X Desired final concentration (\%)}}{100\%}$$

   For 250 ml of a 40% saturated solution of ammonium sulfate, add 100 ml of the saturated ammonium sulfate solution (Product Code A 5479) to 150 ml of concentrated buffer or water.

$$\frac{250 \text{ ml x } 40\%}{100\ \%} = 100 \text{ ml}$$

References

1. Englard, S., and Seifter, S., Precipitation Techniques., Methods Enzymol., **182**, 285-300 (1990).
2. McPherson, A., Crystallization of macromolecules: general principles., Methods Enzymol., **114**, 112-120 (1985).
3. Giegé, R., and Ducruix, A., in Crystallization of Nucleic Acids and Proteins, Ducruix, A., and Giegé, R., eds., Oxford University Press (Oxford, UK: 1999), pp. 1-16.
4. Tessier, P.M., *et al.*, Self-interaction chromatography: a novel screening method for rational protein crystallization., Acta Crystallogr. D (Biol. Crystallogr.), **58** (Pt 10 Pt 1), 1531-1535 (2002).
5. Yaremchuk, A., *et al.*, Improved crystals of *Thermus thermophilus* prolyl-tRNA synthetase complexed with cognate tRNA obtained by crystallization from precipitate., Acta Crystallogr. D (Biol Crystallogr.), **56** (Pt2), 197-199 (2002).
6. Mant, C.T., and Hodges, R.S., in High-Performance Liquid Chromatography of Peptides and Proteins, Mant, C.T., and Hodges, R.S., eds., CRC Press (Boca Raton, FL: 1991), pp. 437-450.
7. Hyder, S. M., *et al.*, in High-Performance Liquid Chromatography of Peptides and Proteins, Mant, C.T., and Hodges, R.S., eds., CRC press (Boca Raton, FL: 1991), pp. 451-475.
8. Jacob, L.R., in Protein Liquid Chromatography, Kastner, M., ed., Elsevier (Amsterdam, Holland: 2000), pp. 235-269.
9. Kent, U.M., Purification of antibodies using ammonium sulfate fractionation or gel filtration., Methods Mol. Biol., **115**, 11-18 (1999).
10. Page, M., *et al.*, in Basic Protein and Peptide Protocols, Walker, J.M., ed., Humana Press (Totowa, NJ: 1994), pp. 407-432.

JM/MAM 10/04

Appendix

Table 1.
Multiplication Factors for Addition of a 100% Saturated Solution of Ammonium Sulfate.

The table below provides multiplication factors for use in calculating the required volume of a 100% saturated ammonium sulfate solution. The table values are independent of temperature.

Desired Final Ammonium Sulfate Percent Saturation

Initial Ammonium Sulfate Percent Saturation	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95
0	0.111	0.176	0.250	0.333	0.429	0.538	0.667	0.818	1.000	1.222	1.500	1.857	2.333	3.000	4.000	5.667	9.000	19.000
10		0.059	0.125	0.200	0.286	0.385	0.500	0.636	0.800	1.000	1.250	1.571	2.000	2.600	3.500	5.000	8.000	17.000
15			0.063	0.133	0.214	0.308	0.417	0.545	0.700	0.889	1.125	1.429	1.833	2.400	3.250	4.667	7.500	16.000
20				0.067	0.143	0.231	0.333	0.455	0.600	0.778	1.000	1.286	1.667	2.200	3.000	4.333	7.000	15.000
25					0.071	0.154	0.250	0.364	0.500	0.667	0.875	1.143	1.500	2.000	2.750	4.000	6.500	14.000
30						0.077	0.167	0.273	0.400	0.556	0.750	1.000	1.333	1.800	2.500	3.667	6.000	13.000
35							0.083	0.182	0.300	0.444	0.625	0.857	1.167	1.600	2.250	3.333	5.500	12.000
40								0.091	0.200	0.333	0.500	0.714	1.000	1.400	2.000	3.000	5.000	11.000
45									0.100	0.222	0.375	0.571	0.833	1.200	1.750	2.667	4.500	10.000
50										0.111	0.250	0.429	0.667	1.000	1.500	2.333	4.000	9.000
55											0.125	0.286	0.500	0.800	1.250	2.000	3.500	8.000
60												0.143	0.333	0.600	1.000	1.667	3.000	7.000
65													0.167	0.400	0.750	1.333	2.500	6.000
70														0.200	0.500	1.000	2.000	5.000
75															0.250	0.667	1.500	4.000
80																0.333	1.000	3.000
85																	0.500	2.000
90																		1.000
95																		