Version 1 Last updated 15 June 2020

ab273568
Ammonium sulfate (saturated solution)

View datasheet: https://www.abcam.com/ab273568
(use https://www.abcam.cn/ab273568 for china, or
https://www.abcam.co.jp/ab273568 for Japan)

For the precipitation of proteins as part of a purification protocol, or for the removal of contaminating small molecules prior to assay.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Ammonium sulfate has been widely used in salting out for protein purification, as it is very water soluble, forms two ions high in the Hofmeister series 1, and has no adverse effects upon enzyme activity. It is generally used as a saturated aqueous solution which is diluted to the required concentration and expressed as a percentage concentration of the saturated solution (a 100% solution). While ammonium sulfate fractionation is a low resolution technique, it is used successfully to reduce the complexity and decrease the sample volume on a daily basis by many scientists.

2. Protocol Summary

Prepare protein sample at desired precipitation temperature

⇩

Add desired amount of ammonium sulfate solution with slow stirring

⇩

Allow to precipitate for 30 minutes to overnight

⇩

Collect precipitated protein by centrifugation at ≥5,000 x *g* for 30 minutes at 4°C

⇩

Resuspend pelleted protein in an appropriate buffer

⇩

If necessary, desalt protein

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store at room temperature, protected from light immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
Ammonium sulfate (saturated solution)	1000 mL	RT

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Stirrer
- High-speed centrifuge (≥ 5000 x g)

8. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

8.1 Ammonium sulfate (saturated solution):

Supplied ready to use. Store at RT protected from light.

9. Sample Preparation

- Compatible with serum, plasma and other body fluids; tissue extracts, animal, plant, bacterial extracts, etc.; cell culture supernatants; fermentation media.

9.1 The protein solution should be equilibrated to the temperature at which the precipitation procedure is carried out.

9.2 There should be no precipitate or debris floating in the solution. Presence of precipitate before addition of ammonium sulfate will not result in reproducible precipitation.

9.3 If required, centrifuge at ≥ 10,000 x *g* at 4°C for 10 minutes.

10. Precipitation Procedure

– Equilibrate ammonium sulfate to the temperature at which the precipitation is to be performed.

10.1 Add the desired amount of saturated ammonium sulfate solution with slow stirring. Allow precipitation for the appropriate amount of time (from 30 minutes to overnight). We recommend that you carry out overnight precipitation at 4°C. Salting out is temperature dependent. Precipitation at 4°C generally needs longer, relative to precipitation at ambient temperature.

10.2 Collect the precipitated protein by centrifugation for 30 minutes at ≥ 5,000 x *g* at 4°C. Decant the supernatant. Dissolve pellet (generally in 10 times lower volume than the initial volume) in appropriate buffer. In some cases, a higher purity can be achieved by re-suspending the pellet in the ammonium sulfate concentration from the previous step and repetition of the centrifugation step. If the target protein is sensitive to the ammonium sulfate pH and the sample does not have sufficient buffering capacity, titrate the pH of the ammonium sulfate appropriately with 10 N NaOH or 6 N HCl before adding to the sample.

10.3 For optimal conditions screening (when working with new target protein), run parallel precipitation experiments at increasing ammonium sulfate concentration (i.e. 10%, 20%, 30%, etc.). After incubation and centrifugation at ≥ 5,000 x g at 4°C for 30 minutes, collect the pellets and analyze for the amount of target protein in all pellets. The final supernatant has very high salt concentration, which can affect biological activity of the target protein (if still present in it). Perform appropriate desalting before analyzing for its biological activity

Δ Note: If there is precipitate in the bottle of saturated ammonium sulfate, warm the solution to 22°C. If the precipitate persists, let the salt settle and use the clear supernatant, as it contains saturated concentration of ammonium sulfate in your environment.

11. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)