ab204708 - Deproteinizing Sample Preparation Kit – TCA

For the removal of macromolecules which might interfere with target metabolites allowing further analysis.

View kit datasheet: www.abcam.com/ab204708

(use www.abcam.cn/ab204708 for China or www.abcam.co.jp/ab204708 for Japan)

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

Storage and Stability:

Store kit at room temperature upon receipt. Kit has a storage time of 1 year from receipt.

Limitations:

Do not use kit or components if it has exceeded the expiration date on the kit labels
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.

Materials Supplied

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Trichloroacetic Acid Solution	3 mL	RT	RT
Neutralization Buffer I	4 mL	RT	RT

Materials Required, Not Supplied

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

- Microcentrifuge
- Microcentrifuge tubes
- Pipettes and pipette tips

Reagent preparation

Briefly centrifuge small vials at low speed prior to opening.

Trichloroacetic Acid Solution: Ready to use as supplied. Store at room temperature, chill on ice before use.

Neutralization Buffer I: Ready to use as supplied. If precipitation is observed, shake bottle a few times to resuspend precipitate Store at room temperature, chill on ice before use.

Sample preparation

Samples do not require additional preparation. Simply follow the sample preparation procedure described in the protocol you are using and perform the deproteinization step when indicated.

Assay procedure

Ensure Trichloroacetic Acid Solution and Neutralization Buffer I are cold prior starting the protein precipitation procedure.

Protein Precipitation:

1. Samples with high protein concentration (e.g. serum, urine):
2. Mix 100 µL sample with 15 µL of cold Trichloroacetic Acid Solution in a microcentrifuge tube.
3. Keep the sample on ice for 15 minutes.
4. Centrifuge at 12,000 x *g* for 5 minutes. Carefully transfer supernatant to another tube,
5. Samples with protein concentration < 25 mg/mL (e.g. tissue lysate, cell lysate, and yeast lysate),:
6. Mix 150 µL sample with 15 µL of cold Trichloroacetic Acid Solution in a microcentrifuge tube.
7. Keep the sample on ice for 15 minutes.
8. Centrifuge at 12,000 x g for 5 minutes. Carefully transfer supernatant to another tube.

NOTE: *Procedure can be stopped at this point and deproteinized samples can be stored at -70°C for up to one month if necessary. We recommend nevertheless to complete the procedure and analyze the samples as soon as possible.*

Sample Neutralization:

1. To neutralize excess Trichloroacetic Acid Solution, add 10 µL of cold Neutralization Buffer I to the collected supernatant. Mix well.
2. Vent sample tube as there may be formation of CO_2. Place sample on ice for 5 minutes.
   Samples are now deproteinized, neutralized and can be directly used in a variety of assays.

NOTE: *For further sample optimization, if reaction buffer capacity is 100 mM or stronger, samples must be diluted (ie. 50 µl of sample diluted to 100 µL with water) to reduce the buffering capacity and ensure optimum pH is reached during reactions.*

Calculations

Addition of Trichloroacetic Acid Solution and Neutralization Buffer I dilutes samples with high protein concentrations down to 80% compared to the original concentration. Correct values in your final calculations using 1.25 as your dilution factor.

(D=125 µl final sample volume /100 µl of sample= 1.25)

Addition of Trichloroacetic Acid Solution and Neutralization Buffer I dilutes samples with low protein concentrations down to 86% compared to the original concentration. Correct values in your final calculations using 1.167 as your dilution factor.

(D=175 µl final sample volume /150 µl of sample= 1.167)

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)