SDS removing buffer

From Gene Bio-Application Ltd.

Cat. No.	Description
PDS010	**SDS removing buffer, 30 ml**
PDS020	**SDS removing buffer, 60 ml**

Storage: Upon arrival, store at 4°C. Shipped at ambient temperature.
This product is guaranteed for one year from date of purchase when properly handled and stored.

SDS removing buffer designed for removing bound-SDS from protein sample using *ProteoConD* kit.

Concentration Protocol using *ProteoConD* kit and SDS removing buffer

1. **Resuspend *ProteoConD* beads by vortexing for 1 min. Transfer the desired amount of beads from the *ProteoConD* tube to a fresh 1.5 ml microcentrifuge tube. For 1 µg of protein take 3 µl of *ProteoConD* beads.**

   For example, take 30 µl of ***ProteoConD*** beads for 10 µg of protein sample.

2. **Equilibrate the *ProteoConD* beads by adding 500 µl of buffer WB*D*; mix the tube vigorously by vortex for 10 seconds.**
3. **Pellet the beads by centrifuge the tube at +4°C for 1 min at 5,000 RPM. Remove the solution without disturbing the *ProteoConD* beads pellet.**
4. **To bind the protein, apply the solution containing the protein sample to the equilibrated *ProteoConD* beads obtained in Step 3.**
5. **Shake the tube containing the protein sample from Step 4 for 30 min at +4°C. To insure maximum binding, mix gently by inverting the tube 4 times every 5-10 min during the incubation.**

   To keep protein activity for downstream protocols, it is recommended to perform all steps of the concentration protocol at +4°C.

6. **Place a *ProteoCon* column in the provided 2 ml collection tube.**

7. **Apply the sample from Step 5 to the *ProteoCon* column, and centrifuge at +4°C for 1 min at 5,000 RPM.**

   The maximum volume of the concentration column is 700 µl. For sample volumes of more than 700 µl, simply load and spin again.

8. **Discard flow-through and place the *ProteoCon* column in the same collection tube.**

   Collection tubes are re-used to reduce plastic waste.

   Binding monitoring: TCA-precipitate the flow-through and save it for an analytical gel (sample 1) to determine whether any unbound protein was remained in the flow-through (TCA precipitation protocol - see handbook).

9. **To wash, add 0.5 ml of WB*D2* buffer to the *ProteoCon* column and mix by tapping on the tube.**

10. **Centrifuge at +4°C for 1 min at 5,000 RPM.**

11. **Discard flow-through and place the *ProteoCon* column in the same collecting tube.**

    Wash monitoring: TCA-precipitate the flow-through and save for an analytical gel (sample 2) to determine whether the protein was release by the washing buffer (TCA precipitation protocol, see handbook).

12. **Repeat Steps 9-11 five times.**

13. **Insert the *ProteoCon* column in a clean 1.5 ml microcentrifuge tube.**

14. **To elute the protein, add 50-100 µl of EB*D* Buffer directly to the *ProteoConD* beads, gently pipette up and down the beads with a 200 µl tip, incubate for 10 min at +4°C. Centrifuge at +4°C for 1 min at 5,000 RPM.**

    Important: If a precipitate is appearing in the EB*D* Buffer tube, incubate the tube at 37°C till the precipitate is disappearing.

    Watch carefully not to damage the filter inside the concentration column tube during the pipetting up and down. The pipetting up and down action will provide better mixing of the elution buffer with the beads. Make sure that the elution buffer is dispensed directly onto the ***ProteoConD*** beads for complete elution of the bound protein.

    Elution monitoring: Re-elute the ***ProteoConD*** beads with 50 µl 2X Protein Loading Buffer (2XPLB) and save for an analytical gel (sample 3) in order to determine whether the protein was not released from the beads by the buffer EB*D* (for recommended elution buffer 2XPLB, see page 12).

    Important: Store the concentrated protein under appropriate conditions. If needed, dialyze the sample using the *GeBAflex-tube* kit from Gene Bio-Application Ltd (see Ordering Information at handbook).