SDS-PAGE Clean-Up Kit

Procedure A for sample volumes of 1 to 100 µL (containing 1–100 µg protein per sample)
Product protocol card

Introduction

Product codes

80-6484-70

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Description

Prior to starting procedure: Chill wash buffer (3) at -20°C for at least one hour. Add reductant to SDS-PAGE sample buffer (7) either 3.1 mg DTT or 5 µL 2-mercaptoethanol per 100 µL.

Step	Duration	Actions
1	15 min	Add 300 µL precipitant (1) to 1–100 µL sample (containing 1–100 µg protein per sample). Vortex. Incubate on ice.
		Add 300 µL co-precipitant (2) and mix.
2	5 min	Centrifuge (at least 12 000 × g).
		Tip: *Position tubes with cap hinge facing outward.*
		Remove supernatant. Centrifuge briefly to bring remaining supernatant to bottom of tube. Remove remaining supernatant with micropipette.
		Tip: *Reposition tubes as before. Proceed rapidly to avoid resuspension or dispersion of pellet. There should be no visible liquid remaining.*
3	5–10 s	Add 25 µL of water to the pellet. Vortex to disperse.
		Tip: *The pellet should disperse but not dissolve*
4	At least 30 min	Add 1 mL chilled wash buffer (3) and 5 µL of wash additive (4). Vortex 20–30 s every 10 min.
		Tip: *Make sure wash buffer is at -20°C before starting. The protein will not dissolve.*
5	5 min	Centrifuge (at least 12 000 × g).
		Tip: *Position tubes with cap hinge facing outward*
6	No more than 5 min	Remove and discard supernatant. Allow pellet to dry.
		Tip: *Do not over-dry pellet.*
7	5 min	Resuspend pellet in 5–40 µL buffer I (5). Vortex and incubate on ice.
8	5–10 min	Add 1 µL buffer II (6) for each 5 µL buffer I used in the previous step. Vortex and incubate on ice.
9	5–10 min	Add an equal volume (6–48 µL) SDS-PAGE sample buffer (7) with reductant. Vortex and incubate at room temperature
		Tip: *If the solution turns yellow, add buffer I in 0.5 µL increments until the solution turns blue.*
10	3 min	Heat at 95–100°C
		Centrifuge briefly and gently tap tube to mix contents.
		Sample is now ready to load on gel

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