Endoplasmic reticulum Purification

Sample (5 g) was ground with a pestle and mortar in grinding buffer containing HEPES, sucrose and KCl. The homogenate was centrifuged at 3,000xg for 10 min at 4°C. The supernatant was removed and centrifuged at 12,000xg for 15 min at 4°C, and then total endoplasmic reticulum or rough endoplasmic reticulum was purified from the resulting supernatant using endoplasmic reticulum enrichment kit (Imgenex, San Diego, CA, USA) as follows. Total endoplasmic reticulum-enriched fraction was purified by centrifugation at 90,000×g for 1 h at 4°C. The resulting pellet was collected as total endoplasmic reticulum-enriched fraction. To purify of rough endoplasmic reticulum -enriched fraction, the supernatant was transferred to a beaker and $CaCl_2$ solution of 15 times the volume of the supernatant was added drop by drop while stirring the supernatant for 15 min. The supernatant with $CaCl_2$ solution was transferred from the beaker to a centrifuge tube and centrifuged at 8,000×g for 10 min at 4°C. The resulting pellet was collected as rough endoplasmic reticulum-enriched fraction.

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