Transport assay with spin-column

Preparation of spin-column

1. Make a small hole at the bottom of the 1.5 ml eppendorf tube with needle (G-18) and make a hole in the lid with a soldering iron.
2. Place the eppendorf tube onto a new eppendorf tube (without holes).
3. Stuff siliconized glass wool at the bottom of the eppendorf tube on the top.
4. Add ca. 1100 ul of Sephadex G-50 fine prepared in 50 mM Tris-Mes buffer (pH 7.5).
5. Spin the column at 2000 rpm for 2 min.
6. Place the eppendorf tube on the top to the new eppendorf tube (without holes).
7. Spin the column again at 2000 rpm for 2 min.
8. After the 2nd spin, ca 500 ul of Sephadex G-50 fine should be kept in the eppendorf tube (Fig. 1).

Transport assay

1. The standard reaction mixture contain, in a total volume of 500 µL, 50 mM Tris-MES buffer (pH7.5), 100 mM KCl, 5 mM MgATP, 50 µM substrate, and membrane vesicles for 100 µg protein.
2. Incubation at 25°C
3. Take 130 µL from the reaction mixture and load it on a Sephadex G-50 spin column
4. Cntrifuged at 2,000 rpm for 2 min.
5. Collect filtrates from the eppendorf tube (bottom) and mix with an equal volume of methanol.
6. Centrifuged at 15,000 rpm for 15 min.
7. Inject aliquots of the supernatants into the HPLC apparatus.

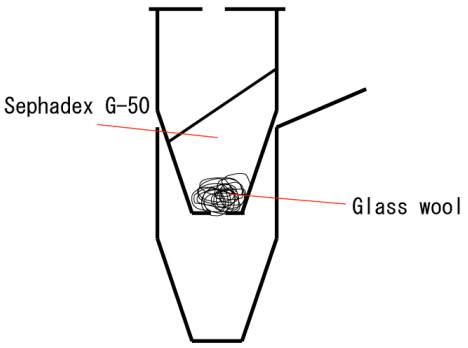

Fig 1

Reference

Otani, M., Shitan, N., Sakai, K., Martinoia, E., Sato, F. and Yazaki, K. (2005)
Characterization of vacuolar transport of the endogenous alkaloid berberine in *Coptis japonica*. *Plant Physiol*., 138, 1939-1946.

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