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ProductInformation

Tubulin
From bovine brain

Product No. **T4925**
Store Temperature –20 °C

Product Description

Tubulin forms microtubules, which consist of tubulin dimers (α and β, 50 kDa each) and microtubule associated proteins (MAPs), primarily MAP1, MAP2 (200-300 kDa), and Tau (50-70 kDa). The microtubules are part of the cellular cytoskeletal system and of the mitosis mechanism. Drugs that interfere with microtubule function, either by promotion of their disassembly (colchicine) or by their stabilization (paclitaxel), will prevent chromosome movement during mitosis and stop cell division.

This product is purified from calf brain by heat-dependent assembly-disassembly cycles. It contains approximately 15% microtubule associated proteins. Each vial contains more than 7.5 mg of protein in assembled form, which will give more than 5 mg of soluble protein after disassembly. The product is supplied as a lyophilized powder containing MES buffer salts, EGTA, EDTA, $MgCl_2$, dithiothreitol, GTP, leupeptin, aprotinin, and sucrose as stabilizer.

Reconstitution

Reconstitute each vial with 900 μl of double distilled water to obtain 1 ml of solution containing 100 mM MES, 1 mM EGTA, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 100 μg/ml sucrose, 1 mM DTT, 0.1 mM GTP, 1 μg/ml leupeptin, 1 μg/ml aprotinin, pH 6.8. The reconstitution should be performed at 37 °C according to the instructions described in the procedure below.

Storage and Handling

The lyophilized tubulin should be stored desiccated at –20 °C. The reconstituted tubulin should be stored in aliquots at –70 °C (see Procedure B, steps 1-2).

Tubulin Assembly Assay

Reagents and equipment

Note: The pH of the solutions is critical, therefore, it should be measured precisely.

A. 0.1 M MES (Product No. M 2933), 1 mM EGTA (E 4378), 0.5 mM $MgCl_2$ (M 0250), 0.1 mM EDTA (ED2SS), 2.5 M glycerol (G 9012), pH 6.50

B. 100 mM GTP (G 8877), pH 7.00

C. 10 mg/ml Paclitaxel (T 7402) in DMSO (D 5879)

D. Two quartz cuvets (1 ml internal volume)

E. Heated spectrophotometer (37 °C) with kinetics capability

F. Tissue grinder, Tenbroeck, 2 ml, Kontes Art. No. 885300-0002

G. 1 ml centrifuge plastic tubes with adaptors (Tubes: Sorvall, CAB, 1 ml Cat. No. O 3103. Adaptors: Sorvall Cat. No. 408).

Procedure A

Note: This procedure is suitable if all the tubulin is to be used within a few hours. Steps 1-3: reconstitution, steps 4-8: disassembly, steps 9-13: GTP induced assembly, and steps 14-15: paclitaxel induced assembly.

1. Bring the vial to room temperature.

2. Add 900 μl of double distilled water at 37 °C.

3. Shake the vial gently at 37 °C approximately 5 minutes until all the material dissolves (solution will be turbid).

4. Incubate the vial on ice for 30-40 minutes.

5. While incubating on ice, homogenize the solution using a 2 ml glass tissue grinder (F) every 10 minutes.

6. Centrifuge the solution in small 1 ml tubes for 40 minutes at 25,000-30,000 x *g* at 4 °C.

7. Collect the supernatant and keep on ice throughout the experiment.

8. Determine protein concentration of the supernatant using Lowry method (expected concentration: 5-6 mg/ml).

9. Set the spectrophotometer: Wavelength: 350 nm, Program: kinetics, Temperature: 37 °C.

10. Fill both control and sample cuvets (preheat cuvets to 37 °C) with 1 ml of buffer A at 37 °C. Zero the spectrophotometer.

11. Dilute supernatant (step 7) 5-fold with Buffer A: remove 200 µl of Buffer A from the sample cuvet, add 200 µl of supernatant, and mix gently by pipetting. Alternatively, dilute the supernatant in warm Buffer A in a tube and transfer to the cuvet.

12. Start to measure the Absorbance at 350 nm.

13. After 2-3 minutes add GTP to a final concentration of 1 mM (10 µl of 100 mM stock solution B). Mix gently by pipetting. The absorbance will increase immediately as a function of microtubule formation and will reach a plateau after 15-30 minutes. The absorbance increase is 0.07-0.15.

14. After 40 minutes add paclitaxel to a final concentration of 20 µg/ml (2 µl of 10 mg/ml stock solution C). The absorbance increases due to polymerization of tubulin that is under the critical concentration for GTP derived polymerization. The absorbance increase will be similar to that obtained in step 13.

15. Monitor the absorbance change for an additional 20 minutes.

<u>Procedure B</u>

<u>Note</u>: This procedure is suitable when the material in the vial is not to be used at once. It is possible to freeze the reconstituted tubulin quickly in liquid nitrogen and store it at –70 °C.

1. Reconstitute a vial of tubulin according to Procedure A, steps 1-3.

2. After reconstitution divide the suspension into 200 µl aliquots (each suitable for one experiment) while keeping the temperature at 37 °C. Freeze the aliquots in liquid nitrogen (do not cool down the material before freezing). Keep the tubulin aliquots at –70 °C.

3. Thaw the frozen tubulin at 37 °C (incubate for a few minutes). If the sample has not been frozen, this step is not needed.

4. Put the 200 µl of sample on ice and add 800 µl of ice cold buffer A.

5. Continue from Procedure A, step 5, with the exception of step 11. The tubulin in Procedure B is already diluted. To prevent condensation on the cuvet, incubate the tubulin sample at 37 °C for 10-20 seconds in a water bath, then transfer the sample to the cuvet preheated to 37 °C.

References

1. Luduena, R.F., et al., Current Opinion in Cell Biology, **4**, 53-57 (1992).
2. Ringel I., and Horwitz S.B, J. Pharmacol. Exp. Ther., **259**, 855-860 (1991).

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