Comparison of the biochemical characteristics of bovine brain and porcine brain tubulin

The past forty years has seen the dominance of bovine brain tubulin as the standard tubulin for biochemical and biophysical studies. Due to recent regulations bovine brain material is no longer available which means it is necessary to use another source that is similar to the bovine version. Cytoskeleton Inc. has introduced a line porcine brain tubulin products that are intended for direct replacement of the bovine version (see Appendix I). These products have performed very similarly if not identical in comparative tests and thus are suitable replacements. The following report substantiates the comparable nature of the two tubulins by several biochemical tests, including:

1. **Polymerization assay** measured by turbidometry
2. **Interaction with motors** and their inhibitors measured by microtubule stimulated ATPase
3. **Interaction with drugs**, efficacy of microtubule inhibitor drugs during polymerization

We hope this comparison will lead to a smooth transition to porcine tubulin, and also be an historical reference point whereby studies on bovine tubulin can be repeated with porcine tubulin without need for extensive testing. If you have any questions or need a datasheet e-mailed to you, contact tservice@cytoskeleton.com .

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Test 1: Polymerization Assay

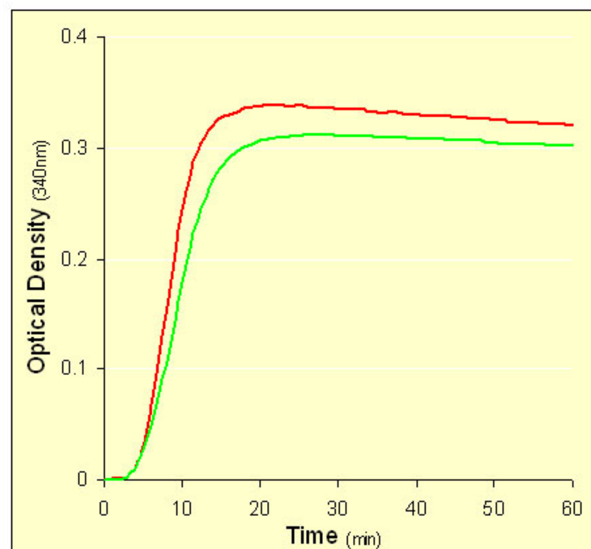
Aim: Compare the rate and extent of polymerization of Cat.# TL238 (bovine) and Cat.# T240 (porcine) tubulins under standard conditions.

Assay conditions: 3.0 mg/ml tubulin
80 mM Pipes buffer pH 6.90 +/-0.05
2 mM MgCl2
0.5 mM EGTA
10 % glycerol

Temperature	37 °C
Volume	100 µl
96-well plate	3696 or 3697 from Corning Costar (half area plate)
Wavelength	340 nm
Readings	Kinetic 60 readings, one per minute.

Assay description: Optical measurement of microtubule formation relies on light scattering by microtubule polymer. Light scatter is equivalent to light absorbance as detected by a normal spectrophotometer, and light scatter is proportional to the concentration of microtubules in the light path. Using this knowledge one can use the regular 96-well plate reader (with 340nm and temperature control capability) to follow the formation of microtubules from tubulin heterodimers. Examples of this assay provided by Cytoskeleton Inc. are BK004P, BK006P and the fluorescence version BK011P.

Figure 1 - Polymerization kinetics of Bovine (red) and Porcine (green) brain tubulin



Results:
Both bovine and porcine tubulin follow a similar profile of increasing optical density over time. They each have a nucleation phase between 0 to 6 min, a polymerization phase 6 to 14 min, and steady state 18 to 60 min.

Conclusions
As both tubulins follow a similar time profile of optical density under conditions that promote polymerization, we can conclude that both tubulins nucleate, polymerize and remain at steady state to a similar extent. Thus experiments which utilize this assay format can interchange bovine for porcine tubulin without need for re-assessing porcine tubulin characteristics.

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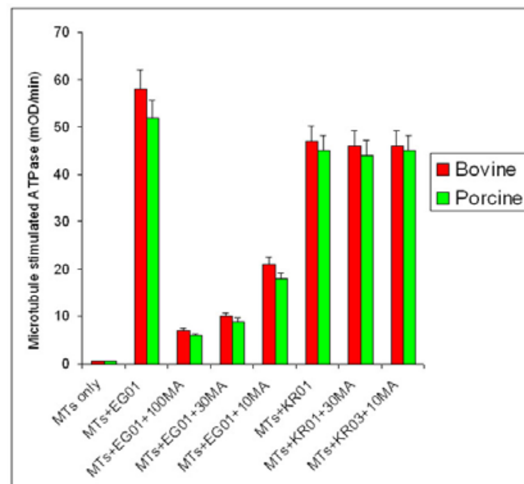
Test 2: Interaction with motors

Aim: Compare the activity of Eg5 and KHC kinesin motor proteins on microtubule stimulated ATPase activity using microtubules made from Cat.# TL238 (bovine) and Cat.# T240 (porcine) tubulins.

Assay conditions:
4 µg Eg5 / assay (Cat.# EG01) or 0.2 µg KHC / assay (Cat.# KR01)
20 µg tubulin as microtubules / assay
15 mM Pipes buffer pH 6.90 +/-0.05
5 mM $MgCl_2$
1 mM ATP
0.5 units phosphonuleotide transferase (detection reagent)
70 µg MESEG (detection reagent)

Temperature	24 °C
Volume	200 µl
96-well plate	269620 Nunc (regular 96-well plate)
Wavelength	360+/-2nm monochromatic (360nm filter will <u>not</u> work)
Readings	Kinetic 40 readings, one per 30s.

Figure 2 – Bovine and Porcine Microtubule stimulated ATPase of Eg5 and KHC in the presence of monastrol.



Results
Two kinesin proteins were compared for microtubule stimulated ATPase activity. Eg5 (Cat.# EG01) is a human mitotic aster associated motor and KHC (Cat.# KR01) is a ubiquitous vesicle transporting motor. The ATPase activity of both these motors was stimulated by the presence of 1uM tubulin as microtubules. Both bovine (red bars) and porcine (green bars) tubulin derived microtubules stimulated the ATPase activity of these kinesins equally. In addition the presence of monastrol, an Eg5 inhibitor, reduced the activity of Eg5 only, not KHC, in the presence of either bovine or porcine microtubules.

Conclusions
Microtubules composed of either bovine or porcine tubulin stimulated two different kinesin ATPase activities. The amount of stimulation was identical between both microtubule species indicating that porcine microtubules can be a direct replacement for bovine microtubules without extensive studies.
The ATPase activity of Eg5 but not KHC can be inhibited with monastrol, this was the same in the presence of either bovine or porcine microtubules which indicates again that porcine microtubules can replace bovine microtubules in kinesin ATPase assays.

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Test 3: Interactions with drugs

Aim: To compare tubulin polymerization kinetics in the presence of vinblastine or taxol using either Cat.# TL238 (bovine) and Cat.# T240 (porcine) tubulin.

Assay conditions:

0 to 30 µM vinblastine
3.0 mg/ml tubulin
80 mM Pipes buffer pH 6.90 +/-0.05
2 mM $MgCl_2$
0.5 mM EGTA
10 % glycerol

Or

0 to 30 µM paclitaxel
1.0 mg/ml tubulin
80 mM Pipes buffer pH 6.90 +/-0.05
2 mM $MgCl_2$
0.5 mM EGTA

Temperature	37 °C
Volume	100 µl
96-well plate	3696 or 3697 from Corning Costar (half area plate)
Wavelength	340 nm
Readings	Kinetic 60 readings, one per minute.

Assay description:

Optical measurement of microtubule formation relies on light scattering by microtubule polymer. Light scatter is equivalent to light absorbance as detected by a normal spectrophotometer, and light scatter is proportional to the concentration of microtubules in the light path. Using this knowledge one can use the regular 96-well plate reader (with 340nm and temperature control capability) to follow the formation of microtubules from tubulin heterodimers. Examples of this assay provided by Cytoskeleton Inc. are BK004P, BK006P and the fluorescence version BK011P.

In the presence of tubulin ligands the kinetics of this reaction are altered, an inhibitor will prolong nucleation times, slow polymerization rate and reduce the extent of steady state. Conversely an enhancer such as paclitaxel will shorten nucleation times, increase polymerization rate and increase the extent of steady state.

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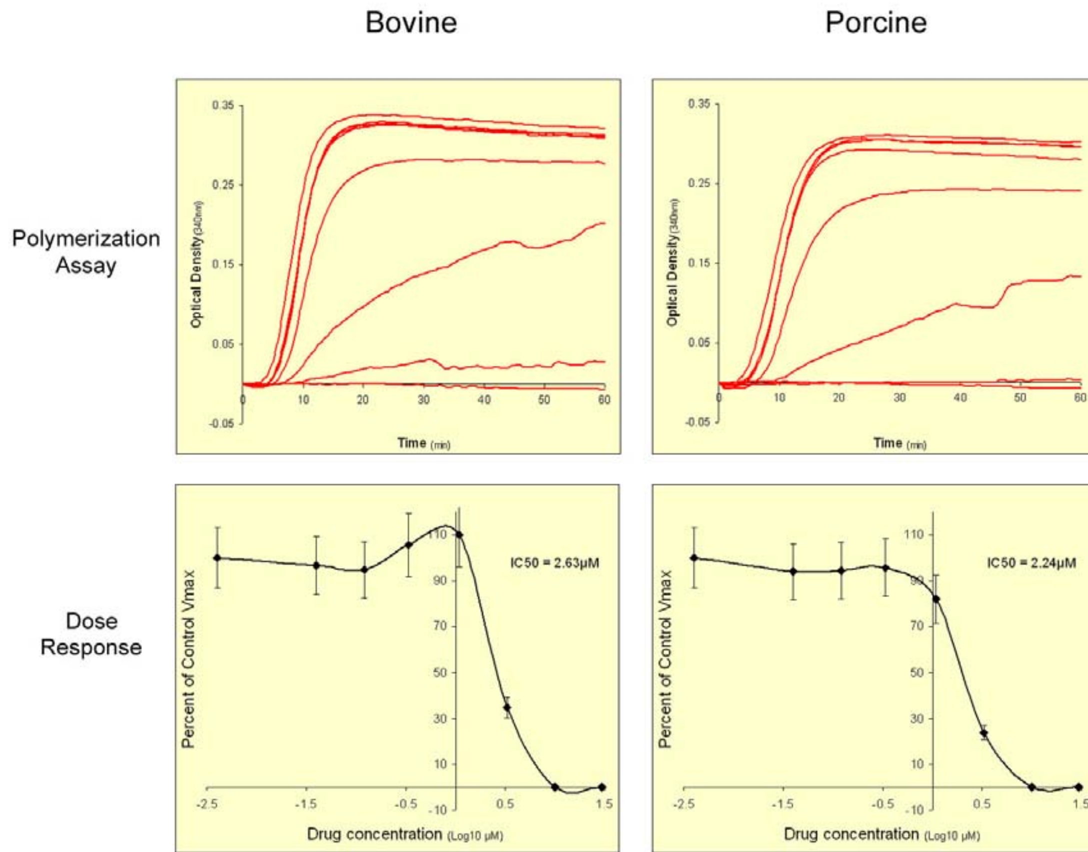
Figure 4 – The effects of vinblastine on the polymerization kinetics of Bovine and Porcine brain tubulins



Results:
Both bovine and porcine tubulins follow a similar profile of increasing optical density over time. They each have a nucleation phase between 0 to 6 min, a polymerization phase 6 to 14 min, and steady state 18 to 60 min. Both tubulins are inhibited by vinblastine to the same extent, with IC50 values of 2.63 and 2.24 µM respectively. The dose response curves have similar structure which indicates both low, medium and high concentrations of drug interact with both tubulins in a similar manor across the concentration range tested.

Conclusions
The effect of vinblastine on tubulin polymerization showed that bovine and porcine tubulin were affected equally. Thus experiments which utilize these tubulins for drug discovery and development (e.g. using Cat.# BK004P, BK006P and the fluorescence version BK011P) can interchange bovine for porcine tubulin without need for re-assessing porcine tubulin characteristics.

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