**Supplemental Figure 1.** SEC-HPLC chromatograms of 8 purified PEGylated rhTSH conjugates

**Supplemental Figure 2.** Tryptic peptide mapping of 40kD SAM conjugate (A) and N-terminal sequencing results of PEGylated tryptic fragments (B). Trypsin (Sequencing Grade, Roche, IN) digested peptides were eluted using a linear gradient of 2–60% B (A: 0.1% trifluoroacetic acid in water, B: 0.08% trifluoroacetic acid in acetonitrile) on a Poroshell 300SB-C8 column over 25 min at 0.4 ml/min at 50°C. PEGylated tryptic fragments, not present in the tryptic peptide map of rhTSH control (data not shown), were collected for automated N-terminal sequence analysis using a Procise protein sequencer (Applied Biosystems, CA). Only 3 tryptic glycopeptides (AT9, AT6, BT3) were detected. Underlined N corresponds to the N-linked glycosylation site.
Supplemental Figure 1

- **10kD MultiSAM**
- **10kD SAM**
- **20kD SAM**
- **40kD SAM**
- **20kD GAM-**
- **40kD GAM-**
- **20kD GAM+**
- **40kD GAM+**
Supplemental Figure 2

Detected sequence | ID  | Expected sequence
-------------------|-----|-------------------
VEXHTAXHXSTXYYY   | AT9 | VENHTACHCSTCYYYH
XVTSESTXXVAK      | AT6 | NVTSESTCCVAK
EXAYXLTIXTTIXAG   | BT3 | ECAYCLTINTTICAG