

density gradient ultracentrifugation and two steps of resource Q (ion-exchange chromatography) using fast performance liquid chromatography (FPLC) system. Ferritin of *P. brevitarsis* is shown to have molecular mass of 600 kDa on a Native PAGE and its subunits consist of two major polypeptide with 27 kDa and 30 kDa presented on a SDS-PAGE. Ferritin was detected by Ferene-S stain and the confirmation of ferritin was also performed by western blotting with polyclonal antibody against Wax moth ferritin. The 27 kDa of *P. brevitarsis* was shown to react intensively with that of Wax moth ferritin whereas the 30 kDa weakly reacted. Other characteristics such as amino acid composition, N-terminal amino acid sequence, and isoelectric point were investigated.

#### **E124** Purification of Transferrin from the Larval Hemolymph of *Galleria mellonella*

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An iron-binding protein has been purified from the last larval hemolymph of *Galleria mellonella* by using rapid purification method. Following the density gradient ultracentrifugation, we purified transferrin-like protein from the hemolymph subphase by immobilized metal ion affinity chromatography (IMAC) using fast protein liquid chromatography (FPLC). This protein was detected by potassium ferricyanide staining method in native polyacrylamide gel and molecular mass was about 80 kDa in SDS-PAGE.

#### **E125** Purification and Characterization of the Ferritin for the Larvae of *Bombyx mori*.

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Ferritin, an iron-storage protein, was partially purified from the hemolymph of *Bombyx mori* by 3 steps, KBr density gradient ultracentrifugation, gel permeation chromatography (Superdex) and reversed phase chromatography (Resource RPC) using fast performance liquid chromatography (FPLC) system. The detection of Fe was performed by Ferene S stain. Native molecular mass of ferritin was estimated as 660 kDa by Native PAGE. The partially purified hemolymph ferritin is composed of 3 subunits and molecular masses of each subunits were determined as about 24 kDa, 26 kDa, and 28 kDa, respectively using SDS-PAGE.

#### **E126** Pak Kinase Activity Regulates Abi Stability

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Abi (Abl interactor) proteins were originally identified as binding partners of the Abl nonreceptor tyrosine kinase. Abi contains serine/threonine-rich regions, proline-rich regions, SH3 domain and PEST motifs involved in protein destabilization. We found that serum starvation resulted in rapid loss of Abi protein. Degradation of Abi is reduced by calpain inhibitors. Overexpression of active Pak mutant, Pak<sup>H83,86L</sup>, inhibits the degradation of Abi upon serum starvation. Activation of Pak by overexpression of active Rac, Rac<sup>G12V</sup>, also inhibits the degradation of Abi upon serum starvation. We also show that multiple bands for overexpressed Abi in Western blots disappear by overexpression of PID (Pak inhibitory domain). Treatment of calpain inhibitor or lactacystin inhibits the degradation of overexpressed Abi. Our results demonstrate that Pak activation stabilizes Abi proteins and suggest a role for Abi in Rac/Pak signaling pathway.