

# ANTIBODIES, BIO THERAPEUTICS & B2B & GENETIC AND PROTEIN ENGINEERING

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## Production of enzymatically active human SKIP for the development of tools to study phosphatidylinositol signaling pathways

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Human SKIP (skeletal muscle and kidney-enriched inositol phosphatase) is a 51 kDa protein found in many tissues including brain, eye, and abundantly in the heart, kidney and skeletal muscle. SKIP exists in the endoplasmic reticulum under resting conditions and translocates to the membrane upon insulin signaling which stimulates protein complex formation involving the insulin receptor. SKIP displays sensitivity towards the signaling lipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub> or PIP<sub>2</sub>) with SKIP hydrolyzing the phosphate at position 5 of the inositol ring of PIP<sub>2</sub>. PI(4,5)P<sub>2</sub> is predominantly located at the plasma membrane where it is involved in protein recruitment and insulin signaling. The purpose of this work is to obtain enzymatically active human SKIP. Previous experiments utilized a fusion protein from pLATE31 SKIP that contained a His-tag. This construct was difficult to purify and produced a low yield of inactive SKIP (as tested via Malachite Green detection of inorganic phosphate). We constructed a new GST-SKIP chimera produced in a heterologous expression system. A similar GST-SKIP species has been reported to have phosphatase activity towards short-chain diC<sub>8</sub> PI(4,5)P<sub>2</sub>. Glutathione affinity chromatography will be utilized to purify soluble GST-SKIP. The detection of the chimera will be conducted by western blot with an anti-GST probe. Fractions enriched with GST-SKIP will be tested for 5-phosphatase activity with both diC<sub>16</sub> and diC<sub>8</sub> PI(4,5)P<sub>2</sub> substrates. Free phosphate detection in SKIP post-reaction mixtures will be carried out using Malachite Green Solution. Confirmation of SKIP activity will lead to removal of the GST-tag and further purification of the target protein to obtain a SKIP preparation for the study of phosphatidylinositol signaling pathways.

### Biography

Somer L Doody is a senior at Utah Valley University, finishing up double Bachelor of Science degrees in Biology and Biotechnology. She has worked on several projects in Molecular Biology and Protein Biochemistry through Innovabio, a contract research organization through Salt Lake Community College in Salt Lake City, Utah. The SKIP project is being done as one of her thesis projects for graduation.

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