Title: Pyruvate Kinase Activity Assay Development

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Mentor- Dr. Pei Zhang (pzhang@liberty.edu)

Student names: Nhat Quang Truong (ntruong1@liberty.edu), Er Yearn Jang (ejang1@liberty.edu), Torvar Castro (jtovar1@liberty.edu)

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Abstract

Pyruvate kinase plays an essential role in living cells. Specifically, pyruvate kinase (PK) is an enzyme that catalyzes the last step in the process of glycolysis and is highly conserved among species. The enzyme transfers the phosphate group from phosphoenolpyruvate (PEP) to ADP, thereby generating one molecule of ATP and pyruvate. Pyruvate kinase has various gene analogs and isoforms in tissues: L (liver), R (red blood cells), M1 (muscle, heart, and brain), and M2 (early fetal tissue). Pyruvate kinase deficiency (PKD) may result in the premature destruction of red blood cells (hemolytic anemia). PKD is the most common inherited cause of non-spherocytic hemolytic anemia. The physiological significance of pyruvate kinase necessitates methods to determine the rate of pyruvate kinase activity in cells. However, few accessible and affordable PK screening methods are available on the market. We attempt to develop an affordable and sensitive pyruvate kinase activity assay in this proposed study. We will generate recombinant tissue-specific pyruvate kinase, purify them using affinity chromatography, and optimize methods to examine activities using a coupled absorbance assay. Pyruvate kinase activity assay development may provide insights into the activity of pyruvate kinase, facilitate implications for medicinal biochemistry such as PK screening for new-born babies, and offer an inexpensive tool for research and educational purposes.

Author: Nhat Q. Truong, Er Y. Jang, Pei Zhang.