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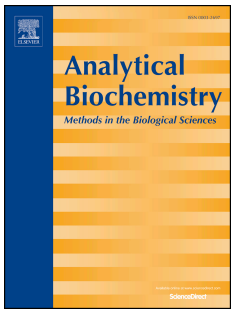

A high-throughput screening assay for pyruvate carboxylase

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A high-throughput screening assay for pyruvate carboxylase

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ABSTRACT

Pyruvate carboxylase (PC) catalyzes the conversion of pyruvate to oxaloacetate (OAA), an important metabolic reaction in a wide range of organisms. Small molecules directed against PC would enable detailed studies on the metabolic role of this enzyme and would have the potential to be developed into pharmacological agents. Currently, *specific* and *potent* small molecule regulators of PC are unavailable. To assist in efforts to find, develop, and characterize small molecule effectors of PC, a novel fixed-time assay has been developed based on the reaction of OAA with the diazonium salt, Fast Violet B (FVB), which produces a colored adduct with an absorbance maximum at 530 nm. This fixed time assay is reproducible, sensitive and responsive to known effectors of *Rhizobium etli* PC, *Staphylococcus aureus* PC, and *Listeria monocytogenes* PC, and is highly amenable to high-throughput screening. The assay was validated using a plate uniformity assessment test and a pilot screen of a library of 1,280 compounds. The results indicate that the assay is suitable for screening small molecule libraries to find novel small molecule effectors of PC.

KEYWORDS
pyruvate carboxylase; diazonium salt; Fast Violet B; oxaloacetate; high throughput screening