

# TAN A: A FLUOROGENIC PROBE FOR THIAMINASE I ACTIVITY

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## ABSTRACT

Vitamin B<sub>1</sub> plays important role in cell metabolism. Deficiency of vitamin B<sub>1</sub> can cause ailments in living organism, including EMS (Early Mortality Syndrom) in the predatory fish salmon in the Great Lakes. Deficiency of vitamin B<sub>1</sub> can be caused by excessive intake of thiaminase I, which is contained excessively in major forage fishes in the Great Lakes, is a transferase-type enzyme that can degrade vitamin B<sub>1</sub> by S<sub>N</sub>(AE) mechanism. However, the occurrence of EMS in the Great Lakes has a year to year variation as large as 90%. In order to understand the pattern of EMS occurrence, a method for thiaminase I activity measurement is needed.

A radiometric assay is accurate and has been used for over 30 years, however it is expensive and requires labs to be permit for radioactive material. Another assay is the 4-NTP colorimetric assay, but it suffers from accuracy problems.

For these reasons, a fluorophorogenic thaimine analogue is presented as a fluorescent probe for Thiaminase activity. The emission of the fluorophore is quenched by photoinduced electron transfer (PET) to the N-substituted pyridinium portion of the probe. Action of the enzyme releases the free pyridine group causing a substantial increase in fluorescence and the measured thiaminase I activity (based on initial rates) was proportional to enzyme concentration up to 60 ng/assay.