A new microassay for the determination of alkaline phosphatase activity in early mouse concepti

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A new enzymatic assay for the determination of alkaline phosphatase (APase) activity in preimplantation mouse concepti is described. This method allows estimation of APase activity of concepti extracts using the fluorogenic substrate, 3,6-fluorescein diphosphate (FDP). For measuring APase activity, 0.1% Nonidet P-40 was used to solubilize the enzyme. Control assays showed that this procedure does not modify the enzyme activity. According to the K(m) obtained for APase from mouse concepti (between 1-2 ?M), the initial concentration of FDP was 20 ?M, which is 10 fold the Km. The assay sensitivity allows continuous recording of the product generated and a reliable determination in less than 20 min. Results show that APase activity in mouse concepti may be detected from the 2-cell stage, increasing exponentially towards the blastocyst stage.