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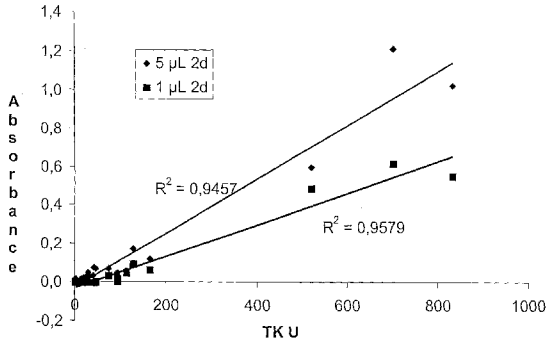
(54) **Title:** A METHOD AND KIT FOR DETERMINATION OF THYMIDINE KINASE ACTIVITY AND USE THEREOF



(57) **Abstract:** A method and assay kit for determination of thymidine kinase (TK) activity in a biological sample, such as blood, serum, plasma, Cerebral Spinal Fluid (CSF), pleural fluid, ascites, tissues, cells and extracts thereof, is described. The method comprises contacting, in a buffer, a Basic Reaction Mixture comprising: solid surface-attached primer and/or template, a modified deoxy nucleoside, such as BromodeoxyUridine, IododeoxyUridine, Fluorodeoxy- Uridine or VinyldexoyThymidine as a kinase enzyme substrate, a phosphate donor, a nucleotide polymerizing enzyme, and a kinase enzyme source devoid of TK activity, such as a yeast extract, with the biological sample. After incubation the amount of modified deoxy nucleoside that has been incorporated into the solid surface-attached primer and/or template, is determined and the TK activity present in the biological sample is directly proportional to the amount of incorporated modified deoxy nucleoside. The method and assay kit are useful in the diagnosing, prognosis monitoring of disease progression and treatment effects of cell-proliferation disorders or diseases, such as cancer, and in the screening of compounds, e.g. new drug candidates, affecting enzymatic pathways, which may obstruct the formation of thymidine phosphates or interfere with nucleic acid synthesis.

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