Method of determining the activity of enzymes converting cytosine derivatives to uracil derivatives in cells, tissues and organisms

Introduction:
Nucleoside analogs are widely used drugs in the chemotherapy of malignant and viral diseases. Their application and effectiveness is considerably influenced by a wide range of mechanisms involving transport, phosphorylation, catabolism and as well mutual competition with natural nucleosides. Cytosine specific enzymes participate in the deamination of analogues of deoxycytidine and their monophosphates into uracil derivatives. Important examples of such analogues are some medicaments, e.g. ara-C (1-β-arabinofuranosylcytosine), dFdC (2',2'-difluoro-2'-deoxyuridine), PSI-6130 (β-D-2'-deoxy-2'-C-methylcytidine), L-dC (β-L-2' deoxycytidine) or 5-aza-dC (5-aza-2'-deoxycytidine). In this respect, it is expected that their deamination could influence the results of a treatment. Currently, however, there is a lack of quick and reliable procedures for determining the activity of cytosine deaminases. The most common approach for determining the activity of cytosine deaminases in cells is the analysis of the products of deamination after the disintegration of the cells, or tissues. However, when using current techniques, it is a relatively long process, often with the usage of radioactive markers and special equipment, which allows the acquisition of data on the average activity in a relatively large population of cells, but not in small cell populations, or in individual cells.

Technology description:
The developed method allows a quick determination of the activity of enzymes transforming cytosine derivatives into uracil derivatives in a sample of tissues, or cells using analogues of cytosine nucleosides. These substances are transformed into uracil derivatives by deamination, and are subsequently detected in DNA or RNA. The amount of the uracil nucleoside analogue is then determined, and the signal can be analyzed by i.e. classical microscopic techniques, flow cytometery or plate readers.

Key features:
- Less than 5 hours necessary for the protocol performance including 4-hour incubation step with the substrate
- Simple performance of the experiment
- Detection of the signal can be performed by three different methods: fluorescence microscopy, plate readers and flow cytometry
- No need for additional development

Development status:
The method has been developed for the use in solutions to allow detection of deaminase activities e.g. in sera or cell suspensions.

IP protection:
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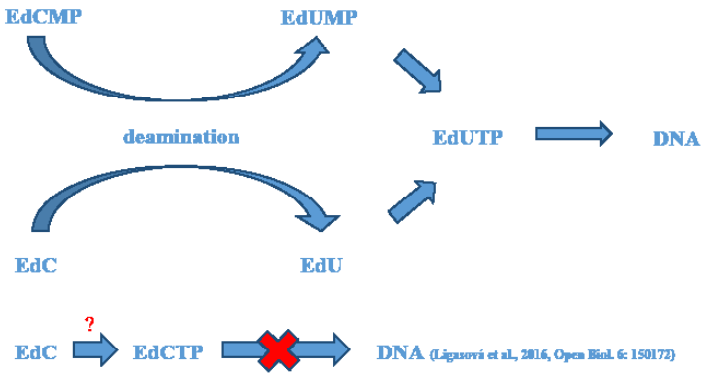
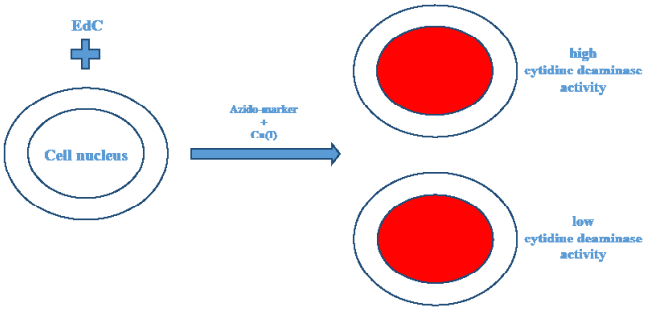
Commercial offer:
Exclusive/non-exclusive license to the patents, related know-how and data

Ownership:
Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague Institute of Applied Biotechnologies, a.s.

Scheme of EdC conversion into EdUMP. As EdCTP is not the effective substrate of the replication machinery in human cells, the detection can be performed by the click chemistry approach.





Simplified scheme of the determination of cytidine deaminase activity in cells.