5-Fluorouracil

Nucleobase analog for in vitro and in vivo cytotoxicity assays
Catalog # sud-5fu
For research use only
Version # 12C14-MM

PRODUCT INFORMATION

Content:
5-fluorouracil (5-FU) is supplied as one bottle of a colorless, water soluble solution at a concentration of 10 mg/ml, filtered to sterility for customer convenience.
- sud-5fu: 250 mg at 10 mg/ml

Shipping and Storage:
5-FU is shipped at room temperature. Store at room temperature in a cool and dark place.
5-FU is stable for one year when properly stored.

SPECIAL HANDLING
Caution should be exercised during the handling and disposal of this product due to potential toxic, mutagenic and carcinogenic properties. Wear gloves when handling this drug. See Materials Safety Data Sheet (MSDS) for safe handling.

GENERAL PRODUCT USE
5-Fluorouracil is a fluorinated analog of uracil. 5-FU is approved by the U.S. Food and Drug Administration (FDA) for cancer chemotherapy as an antineoplastic, antimetabolic agent. With the development of gene therapy, 5-FU can be used in combination with the uracil phosphoribosyltransferase suicide gene.

BACKGROUND
The cytotoxic effects of 5-FU occur following its conversion (through the de novo pyrimidine pathway) to 5-fluoro-deoxuryridine monophosphate (5-FdUMP). 5-FdUMP is an irreversible inhibitor of thymidylate synthase and hence of DNA synthesis through deoxythymidine triphosphate (dTTP) deprivation.

CHEMICAL PROPERTIES
CAS number: 51-21-8
Formula: C4H3FN2O2
Molecular weight: 130.1
Structure:

MECHANISM OF ACTION
5-Fluorouracil exerts its cytotoxicity mainly following its conversion, by a two-step route, to 5-fluoro-uridine monophosphate (5-FUMP). 5-FUMP is further transformed to 5-FdUMP, an irreversible inhibitor of thymidylate synthase and results in dTTP starvation and subsequent apoptosis (Fig. 1). 5-FU can also follow another enzymatic pathway where it is primarily degraded to nontoxic β-alanine.

5-FU is actively transported into mammalian cells. To avoid systemic toxicity of 5-FU, doses must be minimized. Therefore, InvivoGen has created vectors carrying genes encoding uracil phosphoribosyltransferase, an enzyme found in prokaryotes and lower eukaryotes but apparently absent from mammalian cells. UPRT catalyses the direct conversion of 5-FU to 5-FUMP, whereas in mammalian cells 5-FU is converted to 5-FUMP through the concerted action of two highly regulated enzymes. Therefore, expression of UPRT substantially increases 5-FU cytotoxicity in transfected cells. UPRT encoding genes available from InvivoGen are E. coli upp gene and S. cerevisiae fur gene. Both genes were fused to cytosine deaminase genes to generate the following fusion genes: E. coli codA::upp and S. cerevisiae fcy::fur (for more details visit our website www.invivogen.com).

METHOD

Cytotoxicity assay
1- Seed cells at a density of 1 x 10^3 cells/well in a 96-well microtiter plate containing 100 µl of culture medium.
2- Prepare a set of sterile stock dilutions of the 10 mg/ml solution provided.
3- One day later, add increasing concentrations of 5-FU to the wells. Note: Remember to include a control well without the prodrug.
4- After 5-7 days, wash cells with fresh medium and assess cytotoxicity by trypan blue exclusion using a hemocytometer to quantify the results.

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