

Ganciclovir

Prodrug for the HSV-tk/GCV selection system

Catalog code: sud-gcv

<https://www.invivogen.com/ganciclovir>

For research use only. Not for human use.

Version 20102-MM

PRODUCT INFORMATION

Contents

250 mg of Ganciclovir (GCV)

Storage and stability

- Ganciclovir (GCV) is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, aliquots of GCV are stable for 1 month at 4°C and for 6 months at -20°C when properly stored. Do **not** refreeze.

Quality control

- Purity ≥95% (UHPLC)
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK Blue™ TLR4 cells.

SAFETY CONSIDERATIONS

Ganciclovir exhibits reproductive toxicity. Refer to the safety data sheet for handling instructions.

DESCRIPTION

The prodrug Ganciclovir (GCV), a guanosine analog, is commonly used in molecular biology together with the negative selection marker herpes virus thymidine kinase (*HSV-tk*) gene¹. Numerous publications have cited its use in the selection against random recombination events when homologous recombination for the knockin or knockout of a gene is required¹⁻³. In addition, reports have described the use of GCV in the selective removal of undifferentiated cells during *in vitro* differentiation of embryonic stem cells⁴.

Specifically, GCV is used to exert selective pressure on cells transfected with the "cell suicide gene" *HSV-tk*. Normally, eukaryotic cells can survive in the presence of the non-toxic prodrug GCV. However, upon expression of *HSV-tk*, GCV is converted to GCV-monophosphate by HSV1-TK and further phosphorylated to the diphosphate and triphosphate forms by host kinases. GCV-triphosphate, a lethal toxin, is incorporated into the DNA of replicating eukaryotic cells causing premature DNA chain termination and apoptosis⁵.

Other reported uses of GCV include restoring T cell function⁶ and for assessing sensitivity to antiviral treatments⁷. Of note, GCV is approved by the FDA as an antiviral therapy against cytomegalovirus infections.

1. Tamura R. *et al.*, 2020. Gene therapy using neural stem/progenitor cells derived from human induced pluripotent stem cells: visualization of migration and bystander killing effect. *Hum Gene Ther.* 31:352-66. 2. Schwartz F. *et al.*, 1991. A dominant positive and negative selectable gene for use in mammalian cells. *PNAS* 88(23):10416-20. 3. Converse A. *et al.*, 2004. Counterselection and co-delivery of transposon and transposase functions for sleeping beauty-mediated transposition in cultured mammalian cells. *Biosci Rep.* 24:577-94. 4. Naujok O. *et al.*, 1991. Selective removal of undifferentiated embryonic stem cells from differentiation cultures through HSV1 thymidine kinase and Ganciclovir treatment. *Stem Cell Rev Rep.* 6(3):450-61. 5. Moolten F., 1986. Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy. *Cancer Res.* 46:5276-5281. 6. Chang C.M. *et al.*, 2013. *In vitro* treatment with Ganciclovir restores the functionality of exhausted T cells from cancer patients. *Int. J. Gerontol.* 7:171-6. 7. Oon C. *et al.*, 1999. Hepatitis B virus variants with lamivudine-related mutations in the DNA polymerase and the 'A' epitope of the surface antigen are sensitive to ganciclovir. *Antiviral Res.* 41:113-8.

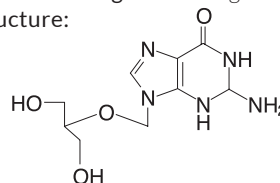
CHEMICAL PROPERTIES

CAS number: 82410-32-0

Formula: C₉H₁₃N₅O₄

Molecular weight: 255.2 g/mol

Structure:



METHOD

Reconstitution of Ganciclovir (GCV):

1. Add 20 ml of distilled water and adjust to pH 12 with NaOH 1M.
Note: GCV is only soluble at pH ≥12.
2. Lower pH to 11 with HCl 1M then add water to bring the total volume to 25 ml of GCV solution (10 mg/ml).
Note: Do not use bacteriostatic water for injection containing parabens which is incompatible with GCV and may cause precipitation.
3. Sterile filter the solution using a 0.22 µm sterile filter.
4. Prepare 1 ml aliquots of GCV and store at 4°C or at -20°C.
5. Thaw frozen aliquots only once (do **not** re-freeze).

Cytotoxicity assay:

1. Seed cells at a density of 1 x 10³ cells/well in a 96-well plate containing 100 µl of culture medium.
2. Prepare sterile stock dilutions of the 10 mg/ml GCV solution.
3. Following an overnight incubation, add increasing concentrations of GCV to the wells.
Note: Include a control well without the prodrug.
4. After 5-7 days, wash cells with fresh medium and assess cytotoxicity using the method of your choice such as the trypan blue dye exclusion assay.

RELATED PRODUCTS

Product	Description	Cat. Code
5-Fluorocytosine	DNA Synthesis Inhibitor	sud-5fc
5-Fluorouracil	DNA Synthesis Inhibitor	sud-5fu
pSELECT-zeo-HSV1tk	Plasmid encoding HSV1-tk	psetz-hsv1tk

TECHNICAL SUPPORT

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