Chloroquine

Endosomal acidification and autophagy inhibitor - InvitroFit™

Catalog code: tlrl-chq-4

https://www.invivogen.com/chloroquine

For research use only

Version 23L08-MM

PRODUCT INFORMATION

Contents

- 4 x 250 mg Chloroquine (diphosphate salt) - InvitroFit™

Storage and stability

- Chloroquine is shipped at room temperature. Store at room temperature (15-25 °C). Protect from light.
- Upon resuspension, chloroquine should be stored at 4 °C. The reconstituted product is stable for 1 month at 4 °C.

Quality control

- Purity ≥ 98% (UHPLC)
- The inhibitory activity has been validated using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Chloroquine is a widely used inhibitor for studying autophagy and the role of endosomal acidification in cellular processes (i.e. intracellular TLR signaling). As a weak base, chloroquine passively diffuses into the acidic compartments of the cell, including endosomes, Golgi vesicles, and lysosomes, where it becomes protonated, trapping it within the organelle. This accumulation of chloroquine leads to an increase in the pH of the compartment and the inhibition of several enzymes that require an acidic pH for proper functioning. Thus, chloroquine prevents the maturation and fusion of endosomes and lysosomes[1].

Chloroquine has an extensive range of biological effects and due to its well-studied toxicity profile is one of the only autophagy inhibitors approved for use in the clinic. It impairs the replication of several viruses, including members of flaviviruses, retroviruses, and coronaviruses, by inhibiting the necessary endosome acidification upon endosomal-mediated viral entry and vesicle trafficking in the later stages of infection (i.e. through the ER-Golgi and exocytosis from the cell)[2,3].

Additionally, the accumulation of chloroquine in lymphocytes and macrophages decreases the production of pro-inflammatory cytokines, specifically TNF-α, and results in anti-inflammatory properties[2]. Specifically, it has been shown, *in vitro* and *in vivo*, that chloroquine has a detrimental effect on the basal autophagic flux by decreasing autophagosome-lysosome fusion due to the impaired function of essential hydrolases[4].

1. Ducharme,J. & Farinotti,R., 1996. Clinical pharmacokinetics and metabolism of chloroquine. Focus on recent advancements. Clin Pharmacokinet 31:257-274. **2. Savarino A. *et al.*, 2003.** Effects of chloroquine on viral infections: an old drug against today's diseases? Lancet Infect Dis 3:722-727. **3. Wang M. *et al.*, 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coro**navirus (2019-nCoV) in vitro. Cell Res 30:269-271. **4. Mauthe M. *et al.*, 2018.** Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. Autophagy 14(8):1435-1455.

CHEMICAL PROPERTIES

Synonym: N4-(7-Chloro-4-quinolinyl)-N1,N1-dimethyl-1,4-pentane diamine diphosphate salt

CAS number: 50-63-5

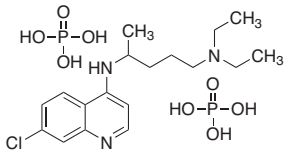
Formula: $C_{18}H_{26}ClN_3 \bullet 2H_3PO_4$

Molecular weight: 515.86 g/mol

Solubility: 50 mg/ml in water

Working concentration: 10-100 µM

Chemical structure:



METHODS

Preparation of stock solution (100 mM)

1. Add 4.846 ml of water to 250 mg of Chloroquine.
2. Vortex until completely dissolved.
3. Filter sterilize and store at 4 °C.

Inhibition of CpG-ODN-meditated TLR9 activity

Below is a protocol using InvivoGen's HEK-Blue™ hTLR9 cells for studying the inhibition of human TLR9 (hTLR9) signaling by Chloroquine. These cells express an inducible secreted embryonic alkaline phosphatase (SEAP) reporter to readily measure the activation of the NF-κB pathway. Changes in SEAP expression due to inhibition of TLR9 signaling can be assessed using QUANTI-Blue™ Solution, a SEAP detection reagent.

For more information, visit https://www.invivogen.com/hek-blue-tlr9.

1. Add 20 µl Chloroquine (10X conc) per well of a flat bottom 96-well plate.
2. Prepare a suspension of HEK-Blue™ hTLR9 cells (~500,000 cells per ml) in culture medium.
3. Add 160 µl of the cell suspension (~80,000 cells) to each well.
4. Incubate the plate at 37°C in a CO_2 incubator for 3 hours.
5. Add 20 µl (10X conc) of an inducer of hTLR9 signaling (e.g. ODN2006) and incubate the plate at 37°C in a CO_2 incubator for 24 hours.
6. Determine inhibition of TLR9 activity by assessing SEAP expression using QUANTI-Blue™ Solution, a SEAP detection medium.

RELATED PRODUCTS

Product	Description	Cat.Code
Bafilomycin A1	Autophagy Inhibitor	tlrl-baf1
HEK-Blue™ hTLR9 Cells	TLR9 Reporter cells	hkb-htlr9
ODN 2006	Stimulatory CpG ODN	tlrl-2006
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT
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