Background: Chloroquine (CQ) is a lysosomotropic agent with an extensive range of biological effects (1). Historically known for its anti-malarial activity, chloroquine is a widely used biological research tool for studying autophagy inhibition. Research studies demonstrate that chloroquine accumulates in acidic lysosomes and increases the lysosomal pH. This inhibits lysosomal hydrolases and prevents autophagosomal fusion and degradation, which can result in apoptotic or necrotic cell death (1-4). Inhibition of chloroquine-induced apoptosis with the V-ATPase inhibitor bafilomycin A1 has been observed in several cell types (4). Chloroquine also enhances the anti-neoplastic effects of the histone deacetylase inhibitor vorinostat (SAHA) (5).

Chloroquine treatment of cells leads to accumulation of light chain 3-II (LC3-II) (1-3). This autophagy marker resides within autophagosomal membranes during the autophagic process and is degraded upon fusion with lysosomes. Chloroquine inhibition of these fusion events effectively blocks LC3-II degradation.

Molecular Formula: C_{18}H_{26}ClN_{3}•2H_{3}PO_{4}

Molecular Weight: 515.9 g/mol

Solubility: Soluble in water at 50 mg/ml. Poorly soluble in DMSO and ethanol.

Purity: >98%

Background References:

Storage: Store lyophilized at room temperature or in solution at -20ºC, desiccated. Protect from light. In lyophilized form, the chemical is stable for 24 months. Once in solution, use within 3 months to prevent loss of potency. Aliquot to avoid multiple freeze/thaw cycles.

Directions for Use: Chloroquine is supplied as a lyophilized powder. For a 50 mM stock, reconstitute the 150 mg in 5.82 ml sterile dH_{2}O. First add 1 ml dH_{2}O to the tube containing the chemical, vortex, and dispense into a new, larger tube. Repeat this action two or three more times to transfer any residual material. Add additional dH_{2}O to the new tube to bring the volume up to 5.82 ml. Filter sterilize into sterile tube. Utilize a syringe and 0.2 μm syringe filter to minimize sample loss.

Working concentrations and length of treatment can vary depending on the desired effect, but it is typically used at 25-100 μM for 12-48 hr.

Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated (-) or treated with Chloroquine (50 μM, overnight; +), using LC3A/B (D3U4C) XP® Rabbit mAb #12741.

Confocal immunofluorescent analysis of HeLa (upper) and C2C12 (lower) cells, treated with Chloroquine (50 μM, overnight; left), nutrient-starved with EBSS (3 hr; middle), or untreated (right), using LC3A/B (D3U4C) XP® Rabbit mAb #12741 (green) and β-Actin (13E5) Rabbit mAb (Alexa Fluor® 555 Conjugate) #8046 (red). Blue pseudocolor= DRAQ5® #4084 (fluorescent DNA dye).