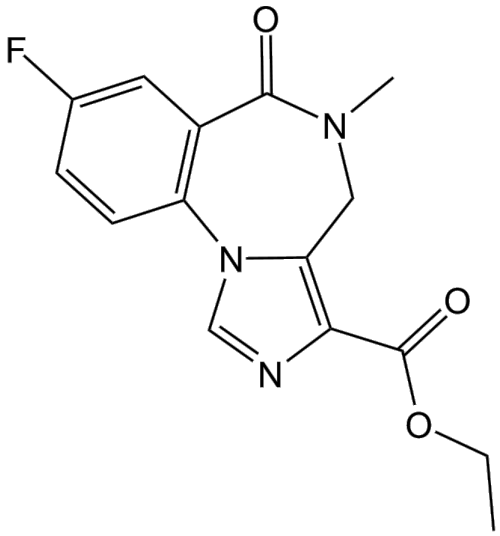
Product Data Sheet

Product Name: Flumazenil
Cat. No.: GC10772

Chemical Properties

Cas. No.	78755-81-4		
Chemical Name	ethyl 8-fluoro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate		
SMILES	CCOC(=O)C1=C2CN(C(=O)C3=C(N2C=N1)C=CC(=C3)F)C		
Formula	$C_{15}H_{14}FN_3O_3$	M.Wt	303.29
Solubility	≥ 15.16mg/mL in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



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Protocol

Cell experiment [1]:	
Cell lines	HEK-293 cells
Preparation Method	HEK-293 cells were cultivated in DMEM medium supplemented with 10% fetal bovine serum, penicillin (100IU/ml) and streptomycin (100μg/ml) at 37°C in an incubator with 5% CO_2. Cells were transfected with α4F, β2 and δ cDNA (1:1:1; α4(F):β2:δ), and cells were co-transfected with 2μg eGFP cDNA for visualization of transfected cells under fluorescence microscopy where the transfection efficiency was consistently 70-80%. The final surface density of plated HEK-293 cells was 10000 cells/plate. Transfected HEK-293 cells were treated with GABA (10μM) plus THP (100nM) or vehicle (0.01% dimethylsulfoxide), at the same time, cells were treated with Flumazenil (10μM) for varying lengths of time (0, 2, 6, 12, 24, and 48h) culminating at the end of the 48h GABA plus THP exposure period. Live cells were probed for surface expression of α4F GABARs under non-permeable conditions which required that all steps be carried out on ice.
Reaction Conditions	10μM; 0, 2, 6, 12, 24, and 48h
Applications	Flumazenil treatment decreased surface expression of α4F GABARs of HEK-293 in a time-dependent manner.
Animal experiment [2]:	
Animal models	Male Albino Swiss mice

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Preparation Method	Male Albino Swiss mice (12 weeks old; 28-31g) were housed in a free-noise room under well-controlled conditions (Room temperature 25±4°C, humidity 48±5%) and were allowed to be acclimatized to those conditions for at least 7 days before any experimental manipulation. The mice were placed in appropriate cages and submitted to free access to drinking water and standardized food pellets. The study groups included a control group that received vehicle, MFA-treated groups at doses of 40mg/kg (i.p.) and group that received Flumazenil (5mg/kg; i.p.) 30 minutes before being injected with MFA. Locomotor activity and catalepsy in mice were analyzed.
Dosage form	5mg/kg; once; i.p.
Applications	Flumazenil treatment mitigated MFA-induced the decreased motor activity and catalepsy in mice.
References:[1] Kuver A, Smith S S. Flumazenil decreases surface expression of α4β2δ GABAA receptors by increasing the rate of receptor internalization[J]. Brain research bulletin, 2016, 120: 131-143.[2] Jarrar Q, Ayoub R, Jarrar Y, et al. Flumazenil pretreatment reduces mefenamic acid-induced central nervous system toxicity in mice[J]. Journal of Integrative Neuroscience, 2023, 22(4): 104.	

Background

Flumazenil is a specific competitive antagonist at benzodiazepine receptors that reverses benzodiazepines-induced sedation[1]. Flumazenil acts by binding benzodiazepine receptors in the central nervous system and competitively blocking benzodiazepine activation of inhibitory GABAergic synapses[2]. Flumazenil has been widely used to reduce acute benzodiazepine withdrawal sequelae and improve psychomotor performance in preclinical models[3].

In vitro, Flumazenil exerts time- and concentration-dependent cytotoxic effects on the MCF-7 cells, with IC_{50} values of 201.8μg/ml, 167.9μg/ml, and 14.37μg/ml at 24, 48, and 72 hours, respectively [4].

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Flumazenil treatment (10μM) for 48 hours reduced the surface expression of α4βδ $GABA_A$ receptors (GABARs) in 3α-OH-5α[β]-pregnan-20-one (THP)-treated HEK-293 cells and increased the endocytosis of the α4βδ GABARs[5]. Incubation with 10μM Flumazenil for 6 hours prevented ethanol withdrawal-induced increases in α4 subunit gene expression and related changes in GABAR function in rat cerebellar granule cells[6].

In vivo, intraperitoneal injection of Flumazenil at a dose of 0.01mg/kg 20 minutes before the start of the experiment markedly alleviated anxiety-like behaviors in BALB/c mice in the elevated plus maze and light/dark box tests[7]. Continuous intraperitoneal injection of Flumazenil (5mg/kg) daily for two consecutive weeks significantly restored the long-term object memory in the mouse model of Down syndrome (Ts65Dn mice) and improved cognitive ability[8]. Intraperitoneal injection of Flumazenil (5mg/kg) 30 minutes before intraperitoneal injection of Mefenamic acid (MFA) reduced MFA-induced central nervous system toxicity, reversed anxiety-like behaviors, restored motor function, decreased seizure intensity, and markedly decreased mortality in mice[9].

References:
[1] Brogden R N, Goa K L. Flumazenil: a reappraisal of its pharmacological properties and therapeutic efficacy as a benzodiazepine antagonist[J]. Drugs, 1991, 42(6): 1061-1089.
[2] Votey S R, Bosse G M, Bayer M J, et al. Flumazenil: a new benzodiazepine antagonist[J]. Annals of emergency medicine, 1991, 20(2): 181-188.
[3] Hood S D, Norman A, Hince D A, et al. Benzodiazepine dependence and its treatment with low dose flumazenil[J]. British journal of clinical pharmacology, 2014, 77(2): 285-294.
[4] TÜRKOĞLU F N, ALPA Ş, GÜLBAHÇE MUTLU E, et al. Determination of Cytoxic Effects of Diazepam and Flumazenil in MCF-7 Cell Line[J]. Diğer, 2023 (İKİNCİ ULUSLARARASI SELÇUK SAĞLIK BİLİMLERİ KONGRESİ).
[5] Kuver A, Smith S S. Flumazenil decreases surface expression of α4β2δ GABAA receptors by increasing the rate of receptor internalization[J]. Brain research bulletin, 2016, 120: 131-143.
[6] Biggio F, Gorini G, Caria S, et al. Flumazenil selectively prevents the increase in α4-subunit gene expression and an associated change in GABAA receptor function induced by ethanol withdrawal[J]. Journal of neurochemistry, 2007, 102(3): 657-666.
[7] Belzung C, Le Guisquet A M, Crestani F. Flumazenil induces benzodiazepine partial agonist-like effects in BALB/c but not C57BL/6 mice[J]. Psychopharmacology, 2000, 148(1): 24-32.
[8] Colas D, Chuluun B, Garner C C, et al. Short-term treatment with flumazenil restores long-term

object memory in a mouse model of Down syndrome[J]. Neurobiology of learning and memory, 2017, 140: 11-16.
[9] Jarrar Q, Ayoub R, Jarrar Y, et al. Flumazenil pretreatment reduces mefenamic acid-induced central nervous system toxicity in mice[J]. Journal of Integrative Neuroscience, 2023, 22(4): 104.