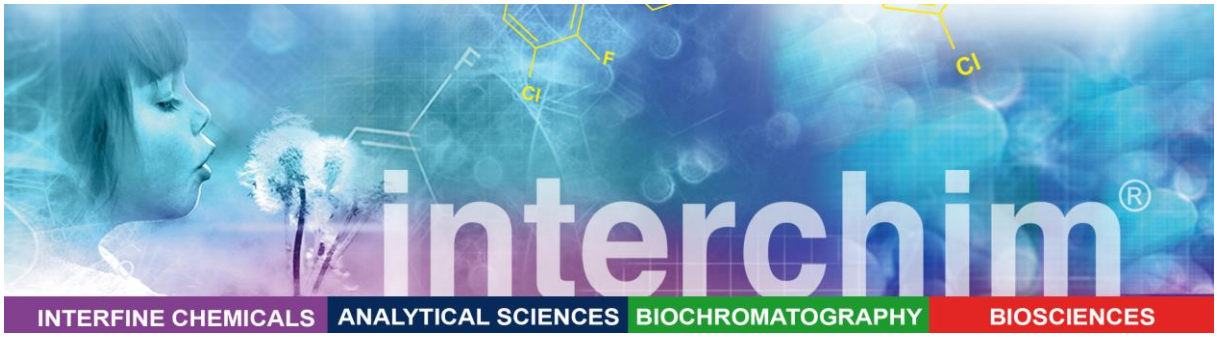
FluoProbes®

FT-486103



BAPTA Ca^{2+} Indicators

Products Information

BAPTA chelators with calcium dissociation constants covering the biologically significant range from 10^{-7} to 10^{-2} M.

Product name Cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}\backslash\lambda_{em}$ max. No Ca^{2+} ε ($M^{-1}cm^{-1}$)	$\lambda_{exc}\backslash\lambda_{em}$ max High Ca^{2+} (nM^{1})	Kd for Ca^{2+}	Soluble in
BAPTA, AM FP-486103, 25mg	764.7	287nm / weak	279 / 363nm [(a)]	- [(a)]	DMSO
BAPTA, K salt FP-453551, 1g	628.88	254nm / weak 5 000[@254nm]	279 / 363nm	Kd (no Mg) 160-590nM, Kd (1 mM Mg^{2}): 700nM []	Water >pH6
BAPTA, Na salt FP-48745A, 1g	564.37	"	"	"	Water >pH6
BAPTA, Cs salt FP-52501A, 1g	1004.03	"	"	"	Water >pH6
MBAPTA AM FP-46778A, 25mg	792.7	287nm /weak	279 / 363nm	- [(a)]	DMSO
MBAPTA, K salt FP-46779A, 100mg	656.87	weak 5 900[@287nm]		(no Mg^{2+}): 40-160nM [depending on buffer]; (1 mM Mg^{2+}): 0.44 µM []	Water
d_iF_{luoro}BAPTA AM FP-46742A, 25mg	800.6	weak	279 / 363nm	- [(a)]	DMSO
d_iF_{luoro}BAPTA, K salt FP-46743A, 100mg	664.8	weak		Kd (no Mg^{2}): 610-635nM, Kd (1 mM Mg^{2}): 705-720nM [depending on buffer]	Water
diBrBAPTA AM FP-48338A, 25mg	922	weak	279 / 363nm	- [(a)]	DMSO
diBrBAPTA, K salt FP-96301A, 100mg	786.7	weak		Kd: 1.6-3.6µM [depending on buffer]	Water
BAPTA FF AM FP-AM934A,10mg	834.5/837	weak	279 / 363nm	- [(a)]	DMSO
BAPTA FF, K salt FP-AM932A, 10mg (eq.Bapta **FT**, **4F**-Bapta)	700.78	weak		65µM []	Water

(a): For AM forms, fluorescence and Ca2+ binding only after hydrolysis []

Storage: **salts** can be stored desiccated and protected from light at room temperature,+4°C[(L)] or -20°C >1 year.
AM esters can be stored desiccated and protected from light at -20°C > 6 months[(M)].

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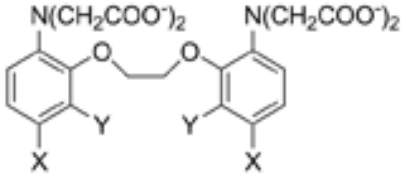
Introduction & technical information



BAPTA is a chelator useful for Ca studies. Different analogs are proposed to cover the biologically significant range of calcium dissociation constants.

Structures/features and applications

• **BAPTA** basic form (X=Y=H) [1,2,-bis(o-aminophenoxy)ethane-N,N,-N',N', tetra-acetic acid], is nonfluorescent and chelates Ca^{2+} very selectively over Mg^{2+}. It is derived from EGTA [ethylene glycol bis(β-aminoethyl ether) N,N,N',N' tetra-acetic acid] to create a selectivity of 10^5 for Ca^{2+} over Mg^{2+}(Tsien 1980). It is better than EDTA and EGTA thanks to a lower pH sensitivity, and ~ 50-400 times faster releases Ca^{2+} ions than EGTA.

These features make BAPTA useful to control the level of both intracellular and extracellular Ca^{2+} in presence of Mg^{2+} concentrations that interfere with other dyes, and is now used essentially in preparation of buffers for Ca^{2+}/Mg^{2+} measurements for buffering calcium.

• The lower affinity fluoro derivatives – **difluoroBAPTA** (X=F, Y=H) and tetrafluoro (**BAPTA FF**, X=Y=F), have been a good source for studying high concentration of calcium using ^{19}F NMR (Murphy 1990).

• The 5,5'-DimethylBAPTA or "**MAPTA**" (X=CH_3, Y=H) is the highest affinity BAPTA compounds of all.

• **DibromoBAPTA** (X=Br, Y=H) has an intermediate affinity and has been used extensively to study calcium mobilization, spatial buffering, and calcium shuttling in many cells.

All the BAPTA products are available as Acetoxymethyl ester, and as free acid form.

• BAPTA indicator as **AM ester** is ***membrane-permeant*** and thus can be loaded into cells by simple incubation of the cells or tissue preparation in a buffer containing the AM ester. Pluronic® F-127, a mild non-ionic detergent, can facilitate AM esters loading. The AM esters themselves do not bind to Ca^{2+}. However, once they have entered the cells, they are rapidly hydrolyzed by intracellular esterases into the parent Ca^{2+} indicators, thus becoming reactive to Ca^{2+}.one can control the cytosolic calcium concentration, an important means to study the roles of calcium. Key advantages of these calcium chelators include relative insensitivity towards intracellular pH change and fast release of calcium.

The BAPTA **free acid salt** forms are ***membrane-impermeant***, but can be loaded into cells via microinjection or scrape loading. They are more commonly used as calcium chelators to form calcium buffers with well-defined calcium concentrations in the biologically significant range from 10^{-7} to 10^{-2} M. The Cs^+ salt of BAPTA has frequently been used for patch-clamp experiments.

See also the literature (references)

Technical tip:

Optimum **calcium concentration range** for an indicator is between 0.1 K_d < [Ca^{2+}] < 10 K_d. BAPTA AM is typically used at 10-100µM. Nearly all chelator molecules are forming the fluorescent complex at high calcium concentration, so no variations are observed in fluorescence intensity. However, there is a big difference in Ca binding (hence signal or trapping) at lower concentrations depending on indicator affinity.

High-affinity chelators (lower K_d) are able to trap calcium very efficiently, so they can buffer calcium response. This attribute is used in the case of the non-fluorescent BAPTA (K_d = 160 nM if there is no Mg^{2+}) to make intracellular calcium concentration zero. A high concentration of BAPTA is loaded along with the desired calcium indicator. In these conditions, all intracellular calcium is sequestered by BAPTA, so fluorescence in absence of calcium can be obtained.

Kd values have been determined in 0.4 µM to ~20 mM in 300 mM KCl for +7 BAPTA analogs [Pethig 1989] (r)

BAPTA AM also inhibits voltage-gated potassium (Kv) channels, including Kv1.3, Kv1.5, and Kv11.1 (Ki = 1.45, 1.23, and 1.30 µM, respectively)[].

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Instructions for use

Handling and Storage

Indicator salts : stock solutions of the salts may be prepared in distilled water or aqueous buffers (pH>6) and stored frozen (<20°C) and protected from light; these solutions should be stable for at least six months.

AM esters should be reconstituted in anhydrous dimethylsulfoxide (DMSO) then used as soon as possible thereafter (within a week) to avoid hydrolysis with subsequent loss of cell loading capacity. DMSO stock solutions of AM esters should be frozen and dessicated and protect from light.

Prepare a stock solution in DMSO at 1mM maximum because of the difficulty to soluble. For a higher concentration, use a surfactant as Pluronic® F127 with a maximum of 0.2% (because of its low toxicity).

To avoid spoiling, the stock solution should be aliquoted and then freezed[‘].

Guidelines for use –in parasites on coverslips [0]

1. Incubation of coverslips overnight with complete. Parasites were resuspended in low calcium Ringer's.
2. Loading of BAPTA-AM into parasites for 10 minutes at 18°C, while BAPTA or EGTA were added to parasites immediately prior to use to minimize leaching of intracellular calcium stores.
3. Parasites were added to coverslips previously washed with low calcium Ringer's and incubated for 15 minutes in a 37°C water bath. After removal from the water bath, coverslips were washed twice with warm low calcium Ringer's and fixed with 2.5% formalin for 20 minutes at 4°C.

Guidelines for use – in muscle fibers [0]

Study of intracellular Ca^{2+} buffers in the regulation of muscle activity

1. BAPTA-AM was dissolved in DMSO to a concentration of 25 mM. The stock solution was then mixed with Ringer solution to provide a final concentration of either 50 or 100 µM.
2. Exposure of fibers to 50 µM BAPTA-AM for 40 min and thereafter reimmersed in the standard Ringer solution for the rest of the experiment.

Other protocols may be found in the literature.

BAPTA #48610

•BAPTA AM Ester #FP-486103 MW: 764.7; CAS:126150-97-8.

•BAPTA Cs salt #FP-52501A MW:1004; CAS: 480436-84-8

•BAPTA Na salt #FP-48745A MW:564.3; CAS: 126824-24-6

•BAPTA K salt #FP-453551 MW:628.80; CAS:73630-08-7.

[]Name: N,N'-[1,2-ethanediylbis(oxy-2,1-phenylene)]bis[N-[2-[(acetyloxy)methoxy]-2-oxoethyl]-1,1'-bis[(acetyloxy)methyl] ester-glycine.

Solubility (K salt): in DMF: 20 mg/ml, DMSO: 20 mg/ml, DMSO:PBS(1:1; pH 7.2): 0.5 mg/ml. [].

Kd (no Mg) 160-590nM, Kd (1 mM Mg^2): 700nM [].

λAbs maxima free/complexed=254nm/weak(274nm)[].

GHS07 | H315, H319, H335 | P261, P264, P271, P280, P302+352, P304+340, P305+351+338, P312, P321, P332+313, P337+313, P362+364, P403+233, P405, P501 [].

[]

- **Aldebaran M.**, *et al*, « Free [Ca^{2+}] dynamics measured in agonist-sensitive stores of single living intact cells: a new look at the refilling process », *The EMBO Journal*, **17,** 1986 (1998) Article
- **Annunziata De Luisi,** *et al*, « Evidence that Ca^{2+} cycling by the plasma membrane Ca^{2+}-ATPase increases the `excitability' of the extracellular Ca^{2+}-sensing receptor », *Journal of Cell Science* **116**, 1527 (2003)
- **Dorval V.,** *et al*, « Regulation of the Phosphotyrosine Content of Human Sperm Proteins by Intracellular Ca^{2+}: Role of Ca^{2+}-Adenosine Triphosphatases », *Biology of Reproduction* **67**, 1538 (2002)
- **Jennie L.** *et al.*, « Intracellular calcium stores in *Toxoplasma gondii* govern invasion of host cells », *Journal of Cell Science* **116**, 3009 (2003) Article
- **Kao, J.P.Y**., *et al.*, “Active involvement of Ca2+ in mitotic progression of Swiss 3T3 fibroblasts », *The Journal of Cell Biology*. **111**, 196 (1990) Article

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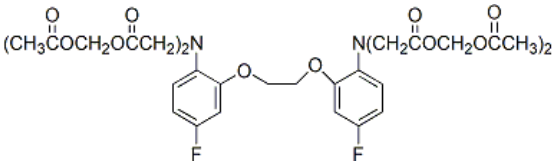
- **Leganés F.** *et al.*, Role of calcium in acclimation of the cyanobacterium *Synechococcus elongatus* PCC 7942 to nitrogen starvation, *Microbiology* 155, 25-34 (2009) Abstract
- **Shumin D.**, *et al*, « $P2X_7$ Receptor-Mediated Release of Excitatory Amino Acids from Astrocytes », *The Journal of Neuroscience*, **23,** 1320 (2003) Article
- **Sun Y.-B.**, *et al.*, « Effects of BAPTA on force and Ca^{2+} transient during isometric contraction of frog muscle fibers »**,** *Am J Physiol Cell Physiol*, **275**, 375 (1998) Article
- **Xinpo J.,** *et al*, « Regulation of a TRPM7-like Current in Rat Brain Microglia », *J. Biol. Chem*., **278**, Issue 44, 42867 (2003) Article

DiFluoro-Bapta #46742

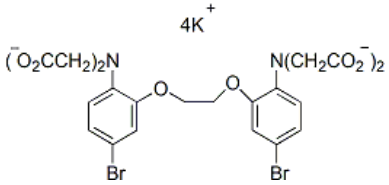


•5,5'-DiFluoro-BAPTA AM Ester #FP-46742A [] MW: 801; CAS: 156027-00-8

•5,5'-DiFluoro-BAPTA K salt #FP-46743A [] MW:665; CAS:152290-47-6

Kd (no Mg^2): 610-635nM, Kd (1 mM Mg^2): 705-720nM [depending on buffer]

DiBromo-Bapta #48338



•5,5'-DiBromo-BAPTA AM Ester #FP-48338A [] MW: 922 []

•5,5'-DiBromo-BAPTA K salt #FP-96301A [] MW: 786.59 []

Kd=1.6-3.6µM [depending on buffer]; Soluble in Water

mBAPTA #46778

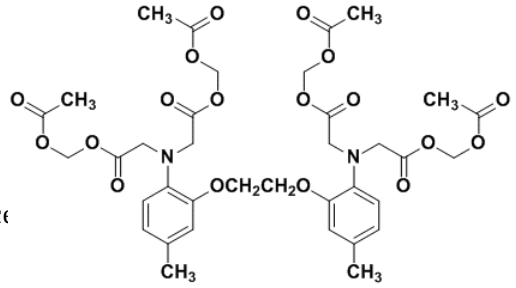
Name : **5,5'-Dimethyl-BAPTA AM ester**

Syn.: 1,2-Bis(2-amino-5-methylphenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl) ester; MAPTA []; MAPTAM []

Catalog Number : **FP-46778A**, 25 mg

CAS: 147504-94-7

Beilstein Registry Number: 8181451



Structure & Properties:

physical — MW= **792.74 g/mol**

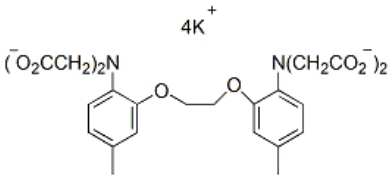
Soluble: DMSO, DMF, Acetonitrile, Ethyl Ace

optical — **Absorption :** λ_{exc} (MeOH) = 287 ± 3 nm; EC= 5 900 ± 500 M^{-1} cm^{-1}

other — **K_d of Ca^{2+}-Binding** : Kd (no Mg^{2+}): 40 to 160nM [depending on buffer]; Kd (1 mM Mg^{2+}): 0.44 µM []

Storage: –20°C (Protect from light and moisture

[]



•5,5'-Dimethyl-BAPTA K Salt #FP-46779A [] MW:656.8; CAS: 73630-09-8.

[]

- **Furuta A.** et al., Microtubule Disruption with BAPTA and Dimethyl BAPTA by a Calcium Chelation-Independent Mechanism in 3T3-L1 Adipocytes, Endocrine Journal, Vol. 56 No. 2:235-243 (2009)
- **González-Flores D.** et al., Nanoceria protects from alterations in oxidative metabolism and calcium overloads induced by TNFα and cycloheximide in U937 cells: pharmacological potential of nanoparticles, Molecular and Cellular Biochemistry Volume 397, Issue 1:245–253 (2014)
- **Winston NJ**, McGuinness O, Johnson MH, Maro B. J Cell Sci 108, 143-151 (1995) [].
- **Fujii C**, Yanagi S, Sada K, Nagai K, Taniguchi T, Yamamura H. Eur J Biochem 226, 243-248 (1994) [].
- **Dubinsky J.M.**, Neurosci. Lett. 150, 129, (1993) [].

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BAPTA FF/TF/4F #AM934-48779

Name :	**BAPTA FF AM ester** **FP-AM934A**, 10 mg
Catalog Number :	5,5',6,6'-Tetrafluoro BAPTA, AcetylMethoxy ester; 1,2-bis(o-Amino-5′-methylphenoxy)ethane-N,N,N′,N′-tetraacetic Acid Tetraacetoxymethyl Ester.
Structure & Properties:	
physical	MW= **834.65 g/mol** **Soluble**: DMSO, DMF
other	**K_d of Ca^{2+}-Binding** : Kd (no Mg^{2+}): 65 µM []
Storage:	–20°C (Protect from light and moisture

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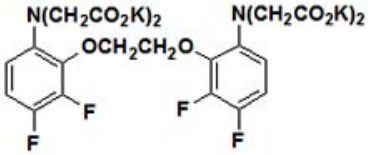

•BAPTA FF salts and free acid are cell impermeable:

BAPTA FF K salt #FP-WU4820 : MW : 700.7 ~~; CAS : ~~ ()

5,5',6,6'-Tetrafluoro BAPTA, tetrapotassium salt (BAPTA FF; TF-BAPTA, 4F-BAPTA)

BAPTA FF free acid #AM932A: MW:548.7. ().

5,5',6,6'-Tetrafluoro BAPTA free acid

Bar-Shir A. *et al*., Single ^{19}F Probe for Simultaneous Detection of Multiple Metal Ions Using miCEST MRI, *J. Am. Chem. Soc*. 137, 78−81 (2015). Article.

Related product(s)

• Associated products

Pluronic® F127, FP-37361A : Nonionic detergent useful for solubilizing hydrophobic compounds to facilitate cell loading.

Coelenterazine, 972333

TPEN, FP-44736A (+): useful tool to identify effects of heavy metals (zinc, iron, copper, manganese, etc.) on fluorescent indicators for other ions.

A-23187, FP-283622 (also known as Calcimycin or Calcium Ionophore III): a calcium ionophore that rapidly equilibrates intracellular and extracellular calcium concentrations. It is commonly used for in situ calibrations of fluorescent calcium indicators.

• Other BAPTA based products:

BAPTA Free Acid, FP-BT547A. MW:476.4; CAS:85233-19-8

5-Methyl BAPTA, Tetramethyl Ester, FP-BT5720. MW: 546/510; CAS:96315-10-5; Building block for calcium indicators.

5-Nitro BAPTA, Free Acid, FP-M1230A; ; MW:521.4CAS: 124251-83-8 [] Kd: 94 (no Mg2)

5-Nitro BAPTA, Tetramethyl Ester, FP-BT5660 MW: 577.54; CAS:172646-43-4; λexc/Em: 382/none (in CH3OH); Building block.

5-Nitro BAPTA, AM ester, FP-BT5670; MW: 809.68; CAS:172646-17-2; [R Pethig, et.el., Cell Calcium 10, 49 (1989)].

5,5'-Difluoro-BAPTA Tetramethyl Ester, FP-AM5021: MW: 568.5

5,5'-Dinitro BAPTA, Free Acid, FP-M1229A. MW: 566.43; CAS:125367-32-0; λexc/Am: 399/none. /ow affinity for Ca2+ (Kd=7.5 mM). Has been used to buffer calcium in cells. [R Pethig, et.el., Cell Calcium 10, 49 (1989)].

5,5'-Dinitro BAPTA, AM ester, FP-AM505A. MW: 622.5; CAS:125367-35-3

5-Formyl-BAPTA, tetramethyl ester, FP-WU5350 MW:560.55;;Building block for calcium indicators.

5-Formyl-5'-Methyl-BAPTA, Tetramethyl Ester [BAPTA-TMFM], FP-BT5640. MW: 574.58; CAS:96315-11-6; Building block for calcium indicators, i.e. Fluo and Rhod; [Grynkiewicz, G., et al.: Journal of Biolgoical Chemistry, 260, 6, 3440 (1985)]

5-Formyl-4-Hydroxy-5'-Methyl-BAPTA, Tetramethyl Ester, FP-BT5600. MW: 590.58; CAS:124903-67-9; Soluble in DMSO, DMF, CHCl3. Building block for calcium indicators, i.e. Fura2

5,5'-'Bisformyl-BAPTA Tetramethyl ester, FP-BT5500. MW: 588.56; CAS: 329789-22-2; Glycine, N,N'-[1,2-ethanediylbis[oxy(4-formyl-2,1-phenylene)]]bis[N-(2-methoxy-2-oxoethyl)-, dimethyl ester; Building block for calcium indicators, i.e. bis-Indo1.

5,5-'Bisformyl-4-hydroxy-BAPTA Tetramethyl ester, FP-WU4700. MW:

4,4'-BisHydroxy-5,5'-BisFormyl-BAPTA, TetraMethyl Ester, FP-BT5510.; MW: 632/620.56; CAS:176767-99-0; Soluble in DMSO, DMF, CHCl3. Building block for calcium indicators, i.e. bis-Fura

5'-Fluoro-5-formyl-BAPTA, TetraMethyl ester, FP-AQRVG. MW: 578.5; CAS:299172-10-4;

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5-Formyl-5'-Nitro BAPTA, Tetramethyl Ester, FP-BT5710. MW: 605.55; CAS:663625-87-4; Soluble in DMSO, DMF, CHCl3. Building block for calcium indicators, i.e. indo-Dextran.

5-Isothiocyano-5'-Methyl-BAPTA, Free Acid, FP-BT5480. MW: 523.52; ; Soluble in DMSO, DMF, CHCl3. Amine-reactive form of BAPTA. Useful for Ca studies with in-situ fixation. (M)

5-Methyl-5'-Nitro-BAPTA #JW7080. [] MW: 536; Kd: 53 (no Mg2)

4-TriFluoroMethyl-BAPTA #FT- 96301A. [] MW: 545; Kd:0.57 (no Mg2)

OG488 BAPTA-1, hexapotassium salt #APIPR0. [same molecule of Oregon Green 488 BAPTA-1].

Ordering information

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