FT-486103

BAPTA Ca Indicators

Products Information

Product name Cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}\backslash\lambda_{em}$ max. Free Ca^{2+} (nm)	$\lambda_{exc}\backslash\lambda_{em}$ max High Ca^{2+} (nm)	mol. abs. ε ($M^{-1}cm^{-1}$)	Kd (nM)	Soluble in
BAPTA, AM FP-486103, 25mg	764.7	287 / weak	279 / 363 [(a)]		- [(b)]	DMSO
BAPTA, K salt FP- 453551, 1g	628.88	254/ weak	279 / 363	[@254 nm] 5 000	160nM	Water >pH6
BAPTA, Na salt FP-48745A, 1g	564.37	254/ weak	279 / 363	[@254 nm] 5 000	160nM	Water >pH6
BAPTA, Cs salt FP-52501A, 1g	1004	254/ weak	279 / 363	[@254 nm] 5 000	160nM	Water >pH6
MBAPTA AM FP-46778A, 25mg	792.74	weak			-	DMSO
MBAPTA, K salt FP-46779A, 100mg	792.74	weak		[@287 nm] 5 900	40nM	Water
d_iF_{luoro}BAPTA AM FP-46742A, 25mg	801	weak			-	DMSO
d_iF_{luoro}BAPTA, K salt FP-46743A, 100mg	665	weak			635nM	Water
diBrBAPTA AM FP-48338A, 25mg	922	weak			-	DMSO
diBrBAPTA, K salt FP-96301A, 100mg	787	weak			1.6µM	Water
BAPTA FF AM FP-AM934A, 10mg	834.5/837	weak			-	DMSO
BAPTA FF, K salt FP-AM932A, 10mg	477	weak			-	Water
TF-BAPTA, K salt FP-B407W0, 25mg	700.78				65µM	DMSO, CH_3OH and H_2O

(a) binding of AM forms occurs after hydrolysis (b) QY: 0.03

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

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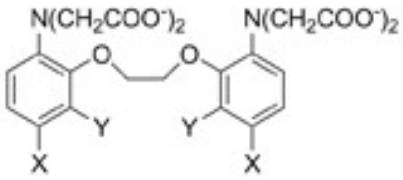
Introduction & technical information

BAPTA, as its basic form [1,2,-bis(o-aminophenoxy)ethane-N,N,-N',N', tetra-acetic acid], is a nonfluorescent chelator and is highly selective for Ca^{2+} 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 105 for Ca^{2+} over Mg^{2+}. It is better than EDTA and EGTA, with lower pH sensitivity, and releases Ca^{2+} ions about 50-400 times faster 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 as chelating agent for the preparation of buffers for Ca^{2+}/ Mg^{2+} measurements.

• **BAPTA** (X=Y=H) itself is now used essentially for buffering calcium.

BAPTA: K_d of Ca^{2+}-Binding : [no Mg^{2+}] 0.59 µM; [1 mM Mg^{2+}] 0.70 µM.

• 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 F NMR.

BAPTA FF, K_d of Ca^{2+}-Binding : [no Mg^{2+}] 65 µM

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

BAPTA FF, K_d of Ca^{2+}-Binding : [no Mg^{2+}] 40mM . 1

• **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.

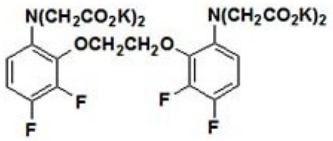
MBAPTA FF, K_d of Ca^{2+}-Binding : [no Mg^{2+}] 1.6µM

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. The Cs^+ salt of BAPTA has frequently been used for patch-clamp experiments. The salt forms are more commonly used as calcium chelators to form calcium buffers with well-defined calcium concentrations (calcium dissociation constants covering the biologically significant range from 10^{-7} to 10^{-2} M).

5,5',6,6'-Tetrafluoro BAPTA, tetrapotassium salt (TF-BAPTA) is a cell impermeable form of BAPTA.



Applications:

See the literature (references)

Optimum **calcium concentration range** for an indicator is between $0,1 \cdot K_d < [Ca^{2+}] < 10 \cdot K_d$. Nearly all chelator is 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 high intracellular chelator concentration can buffer calcium response. This attribute is used in the case of the non-fluorescent BAPTA (K_d = 190 nm if there is no Mg^{2+}) to make intracellular calcium concentration zero and obtain F_{min}. 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.

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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 her 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.

References – BAPTA #48610

- **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)
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- **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

References – TF-BAPTA

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)

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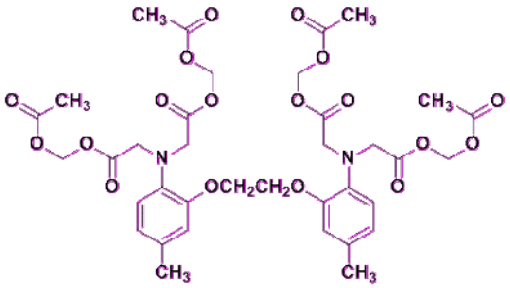
References – mBAPTA #46778

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
Catalog Number :	**FP-46778A**, 25 mg CAS: [147504-94-7]
Structure & Properties:	MW= **792.74 g/mol**
physical	**Soluble**: DMSO, DMF, Acetonitrile, Ethyl Acetate and Chloroform
optical	**Absorption :** $\lambda_{exc\ (MeOH)}$ = 287 ± 3 nm **EC= 5 900 ± 500** M^{-1} cm^{-1}
other	**K_d of Ca^{2+}-Binding** : Kd (no Mg^{2+}): 40nm to 0.16 µM; Kd (1 mM Mg^{2+}): 0.44 µM
Storage:	–20°C Protect from light and moisture

- **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)



References –BAPTA FF #AM394

Name :	**BAPTA FF AM ester** 5,5',6,6'-Tetrafluoro BAPTA, AcetylMethoxy ester
Catalog Number :	**FP-46778A**, 25 mg CAS: [147504-94-7]
Structure & Properties:	MW= **834.65 g/mol**
physical	**Soluble**: DMSO, DMF
other	**K_d of Ca^{2+}-Binding** : Kd (no Mg^{2+}): 65 µM
Storage:	–20°C Protect from light and moisture

Related product(s)

• Associated products

- Pluronic® F127, FP-37361A
- Coelenterazine, 972333

• Other BAPTA based products

- BAPTA Free Acid, FP-BT547A; CAS:[85233-19-8]; MW:476.4
- 5-Methyl BAPTA, Tetramethyl Ester, FP-BT5720
- 5-Nitro BAPTA, Free Acid, FP-M1230A; CAS:[124251-83-8]; MW:521.4
- 5-Nitro BAPTA, Tetramethyl Ester, FP-BT5660
- 5,5'-Difluoro-BAPTA Tetramethyl Ester, FP-AM5021; MW:568.5
- 5,5'-Dinitro BAPTA, Free Acid, FP-M1229A
- 5,5'-Dinitro BAPTA, AM ester, FP-AM505A: MW:622.5
- 5-Formyl-BAPTA, tetramethyl ester, FP-WU5350;
- 5-Formyl-5'-Methyl-BAPTA, Tetramethyl Ester [BAPTA-TMFM], FP-BT5640.
- 5-Formyl-4-Hydroxy-5'-Methyl-BAPTA, Tetramethyl Ester, FP-BT5600.

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- 5,5'-'Bisformyl-BAPTA Tetramethyl ester, FP-BT5500.
- 5,5-'Bisformyl-4-hydroxy-BAPTA Tetramethyl ester, FP-WU4700.
- 4,4'-BisHydroxy-5,5'-BisFormyl-BAPTA, TetraMethyl Ester, FP-BT5510
- 5'-Fluoro-5-formyl-BAPTA, TetraMethyl ester, FP-AQRVG0, CAS:[299172-10-4] MW:578.5
- 5-Formyl-5'-Nitro BAPTA, Tetramethyl Ester, FP-BT5710.
- 5-Isothiocyano-5'-Methyl-BAPTA, Free Acid, FP-BT5480.

Ordering information

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