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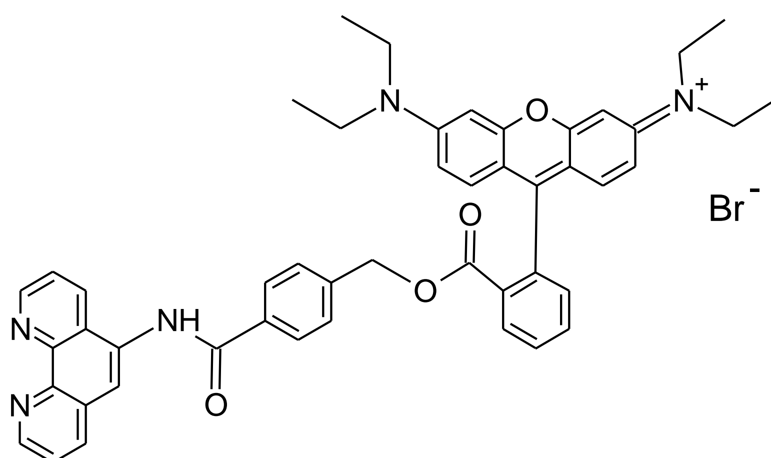
PRODUCT INFORMATION

RPA

Cat. No. ME043.1 (RPA.1) (1 mg)
Cat. No. ME043.2 (RPA.2) (5 mg)

Rhodamine B-[(1,10-phenanthrolin-5-yl)-aminocarbonyl]benzylester



- Fe^{2+} specific fluorescent "sensor"
- Mitochondria specific
- Determination of the mitochondrial chelatable iron pool
- Assessment of mitochondrial iron uptake
- Assessment of alterations of the mitochondrial chelatable iron pool under pathological conditions
- Assessment of the contribution of mitochondrial chelatable iron to physiological and pathological cellular processes
- Assessment of iron reduction

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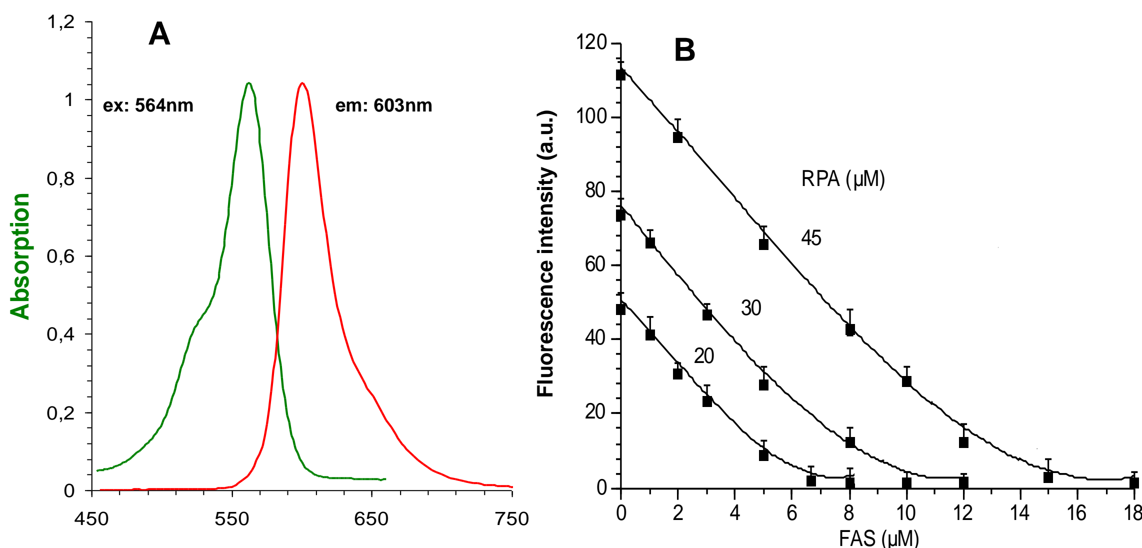
Selective Determination of Mitochondrial Chelatable Iron in Viable Cells with Fluorescent "Fe-Sensors" RPA and RDA

Iron is essential for many biological processes, but is also detrimental as it fosters the generation of highly destructive oxygen species. Mitochondrial chelatable iron is considered to contribute to several human diseases. In the past, attempts to determine the intra-mitochondrial pool of chelatable iron was problematic, due to insufficient mitochondrial accumulation or uniform cellular distribution of iron-selective indicators [Lit 3]. The fluorescent non-toxic "Fe-sensors" RPA and RDA are the first, which allow to determine this iron pool specifically in single intact mitochondria. They are new tools to assess the function of labile ("redox-active") iron e.g. in processes of cell and tissue injury where free radicals are considered to be involved.

Product Information

RPA is used as a selective, high quantum yield fluorescence marker for Fe(II) in biological samples. The cationic fluorophore of the membrane-permeable compound allows the observation by e.g. fluorescence microscopy and, based on the negative membrane potential of mitochondria, the targeting especially into mitochondria of viable cells. In a cell-free system, RPA fluorescence (λ_{max} 601 nm) is strongly and stoichiometrically quenched by Fe^{2+} ions.



A: Absorbance and emission spectra for RPA (20 µM RPA in a "simple buffered solution" (SBS): 2mM ascorbate, 0.15% SDS and 10mM Tris/HCl at pH 8.2) in a cell-free system.

B: Effect of Fe^{2+} on RPA (20-45 µM) fluorescence in "SBS": Fe^{2+}, added as increasing concentrations of ferrous ammonium sulphate/citric acid trisodium salt dehydrate (FAS) from stock solution (1mM) that add. contained 20mM ascorbate, mixed with the medium. Fluorescence (in arbitrary units a.u.) of portions of the medium containing known concentrations of RPA and Fe^{2+} is determined 2 min later, after complete formation of the RPA/Fe^{2+} complex. Zero fluorescence is equal to fluorescence of medium without dye.

RPA selectively accumulates in the mitochondria of e.g. cultured hepatocytes [Lit 1,2]. Intra-mitochondrial RPA fluorescence is quenched when iron is added to cells in a membrane-permeant form. It increases when the mitochondrial chelatable iron available is experimentally

decreased after addition of membrane-permeant transition metal chelators pyridoxal isonicotinoyl hydrazone and 1,10-phenanthroline. This increase of RPA fluorescence allows to specifically quantify mitochondrial chelatable iron in viable cells using ex situ calibration [Lit 1].

Application in a cellular system

RPA can be used as described in Lit. 1. Viable cells cultivated on e.g. coverslips or Pentz chambers are incubated with RPA (0.01-10 µM, prepared from stock solutions of 10 µM or 1 mM in DMSO) for 10-12 min at 37°C in HBBS ("Hanks balanced sot solution"). Cells are washed subsequently three times with dye-free HBBS. To circumvent RPA binding to the chambers and improve mitochondrial loading, cells should be transferred after RPA loading to a second (indicator-free) Pentz chamber and incubated another 15 min. at 37°C. Cells should now be covered with an appropriate medium (e.g. HBBS or L-15 medium for hepatocytes) at 37°C. Intra-mitochondrial RPA fluorescence is determined using e.g. quantitative laser scanning microscopy. The red fluorescence of RPA is excited at $\lambda_{exc.}$ = 543 nm and collected through a long-pass filter near $\lambda_{exc.}$ = 601 nm. Scanning parameters for quantitative and qualitative measurements depend on the microscope system used for the experiments. The intra-mitochondrial level of chelatable iron can be manipulated 5-10 min after starting of the measurements by addition of $FeCl_3$/8-hydroxyquinoline complex (5-15 µM) or membrane permeant iron chelators, e.g. PIH (pyridoxal isonicotinoyl hydrazone, 2mM) or 1,10-phenanthroline (2 mM). For control measurements, parallel cultures are loaded with the iron-insensitive control RPAC (Rhodamine B-[(phenanthren-9-yl)aminocarbonyl]benzylester) containing the same fluorophore. The same loading conditions should be used as described above.

Ex situ calibration

The *ex situ* calibration of intra-mitochondrial RPA concentrations is determined by comparing the mitochondrial fluorescence (in arbitrary units) after "dequenching" with PIH (2 mM) with the fluorescence of RPA standards (5-80 µM) dissolved in a "mitochondrial medium" (composition see Lit 1). In order to obtain a calibration curve, 100 µl portions of medium with known RPA concentrations are placed on the same coverslips used in cellular experiments. The same focal plane and laser scanning parameters used for the quantitative cellular fluorescence measurements are used in the cell-free *ex situ* measurements.

Ex situ calibration of Fe^{2+} induced quenching of RPA fluorescence

1 ml of "mitochondrial medium" (see Lit. 1) is transferred into 1.5 ml tubes, incubated at 37°C. RPA (20-80 µM) is added. Known concentrations of FAS (ferrous ammonium sulphate/citric acid trisodium salt dihydrate) from freshly prepared stock solution (1 mM) with 20 mM ascorbate are added. The RPA/Fe^{2+} is formed during app. 2 min incubation time. The calibration curve is obtained by measuring the RPA fluorescence with the same focal plane and laser scanning parameters used for the quantitative cellular fluorescence measurements and for the *ex situ* calibration.

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Product Data

product name:	RPA
product code:	ME043.1 (1 mg) and ME043.2 (5 mg)
chemical name:	Rhodamine B-[(1,10-phenanthroline-5-yl)-aminocarbonyl]benzylester
molecular formula:	$C_{48}H_{44}N_5O_4Br$
molecular weight:	834g/mol
absorption maximum:	λ_{max} (log ε) = 564 nm (4.95), 510-535 broad shoulder
emission maximum:	λ_{max} 601 nm
stability:	< 4°C, stored dry and protected from light
appearance:	purple solid
purity:	> 97% (^{1}H NMR, 500 MHz)
quenching stoichiometry:	RPA/Fe^{2+}: 3:1 [mol/mol]
in vitro toxicity:	non toxic

Considerations for Use

The product is used as a selective, high quantum yield fluorescence marker for Fe(II) in biological samples, especially in mitochondria of viable cells. Measurements can be performed by fluorescence spectroscopy, fluorescence plate readers, FACS, video microscopy and laser scanning microscopy. 1 mM to 5 mM stock solutions of RPA in DMSO can be prepared and aliquots should be kept at –20˚C in the dark. When stored properly at –20˚C, the solutions can be used for at least 2 – 3 months.

Literature

1. Selective determination of mitochondrial chelatable iron in viable cells with a new fluorescent sensor; F. Petrat et al. *Biochem. J.* (2002) 362, 137-147

2. Cold-induced apoptosis of hepatocytes: mitochondrial permeability transition triggered by nonmitochondrial chelatable iron; U. Rauen et al. Free Radical Biology & Medicine, Vol. 35, No. 12, pp. 1664-1678, 2003

3. The chelatable iron pool in living cells: A methodically defined quantity; F. Petrat et al. Biol. Chem., Vol. 383, pp. 489-502, 2002

4. Assessment of chelatable mitochondrial iron by using mitochondrion-selective fluorescent iron indicators with different iron-binding affinities. U. Rauen et al. ChemBioChem 2007, 8, 341-352

5. Oxidative inactivation of mitochondrial Aconitase results in iron and H_2O_2-mediated neurotoxicity in rat primary mesencephalic cultures. David Cantu *et al.* PlosOne, September 2009, Vol 4, Issue 9, p 1-9

6. Tryparedoxin peroxidase-deficiency commits trypanosomes to ferroptosis-type cell death. M. Bogacz et al.; Cell Biology, July 2018

7. Mitochondrial ferritin limits oxidative damage regulating mitochondrial iron availability: hypothesis for a protective role in Friedreich ataxia. Alessandro Campanella et al. Hum. Mol. Genet. 2009, Jan 1; 18(1): 1-11

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8. Uptake of Non-Transferrin Iron by Erythroid Cells. Eugenia Prus et al. Anemia. 2011; 2011: 945289

9. Chelation of mitochondrial iron prevents seizure-induced mitochondrial dysfunction and neuronal injury. Li-Ping Liang et al. Journal of Neuroscience 5 November 2008, 28 (45) 11550-11556

10. Reduction in mitochondrial iron alleviates cardiac damage during injury. Hsiang-Chun Chang et al. EMBO Molecular Medicine: 8 (3) (2016), 247-267

11. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. Yoshihiko Ichikawa et al. Cardiology. J Clin Invest. 2014 Feb 3; 124(2): 617–630.

12. Iron chelators for treating and preventing cell death and organ damage following an ischemic event. Hossein Ardehali et al. 2016. US20170014397A1, US patent.

13. Mitochondrial iron and energetic dysfunction distinguish fibroblasts and induced neurons from pantothenate kinase-associated neurodegeneration patients. Paolo Santambrogio et al. Neurobiology of Disease, Vol. 81, September 2015, pp 144-153

14. Shawn, the Drosophila homolog of SLC25A39/40, is a mitochondrial carrier that promotes neuronal survival. Jan R. Slabbaert et al. The Journal of Neuroscience 10 February 2016, 36 (6) 1914-1929.

15. Iron regulatory protein deficiency compromises mitochondrial function in murine embryonic fibroblasts. Huihui Li et. al. Nature, Scientific Reports volume 8, Article number: 5118 (2018)

16. Iron-chelator complexes as iron sources for early developing human erythroid precursors. J.M. Leimberg et al., Translational Research 2008, 151:88-96

17. Friedreich’s Ataxia, No Changes in Mitochondrial Labile Iron in Human Lymphoblasts and Fibroblasts. F. Petrat et al., J. biology. Chem. 280, 8, February 25, pp. 6701–6708, 2005

18. Designer Aminoglycosides That Selectively Inhibit Cytoplasmic Rather than Mitochondrial Ribosomes Show Decreased Ototoxicity. Timor Baasov et al., J. biol. Chem. 289/4, pp. 2318–2330. January 24, 2014

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