FT-21527A

Calcium Calibration kit

Buffers to generate Ca^{2+} calibrated solutions for Ca^{2+}-binding indicators measurements

Product information

Catalog #: FP-21527A, 1 kit
Name: **Calcium Calibration kit**

Store at +4°C or at –20°C for long term (M)

This kit contains:
Component A (zero free Ca^{2+}):
50ml of Zero mM CaEGTA (10 mM K_2EGTA,100mM KCI and 10mM MOPS;pH 7.20)

Component B (40uM free Ca^{2+}):
50ml of 10 mM CaEGTA (10 mM CaEGTA,100mM KCI and 10mM MOPS;pH 7.20)

This kit provides a range of calibration buffers with accurate calcium concentrations and can generate calcium concentrations from zero up to 40µM free Ca^{2+}. It is useful for the calibration of fluorescent Ca^{2+} indicators[1,2]. Minimizing Ca^{2+} concentration errors provides accurate Ca^{2+} binding indicator determinations.

The highest Ca^{2+} concentration is 10 mM CaEGTA, which gives a $[Ca^{2+}]$free of about 39 µM. This $[Ca^{2+}]$free is high enough to saturate indicators with Kd values in the 0.1–1 µM range such as fura-2, indo-1, fluo-8, fluo-3 indicators.

$[Ca^{2+}]$free can be calculated according Roger Tsien 1989 method.

Technical information

The dissociation constants (Kd) of fluorescent calcium indicators are a function of temperature, ionic strength and pH. ln order to make accurate calcium measurement, it is therefore important to determine the dissociation constant at a given condition. Our calcium calibration buffer kit is specifically designed for easy calibration of calcium indicators by providing known free calcium concentrations ranging from zero up to 40 uM.
ln theory, any desired free calcium concentration between zero and 40 uM can be obtained by simply mixing different ratio of components A and B as indicated by the following formula:

$[Ca^{2+}]free = Kd^{EGTA} X ([CaEGTA]/[K_2EGTA])$

where Kd^{EGTA} is the dissociation constant of CaEGTA and its value is also a function of temperature, ionic strength and pH. For your convenience, listed in table are Kd^{EGTA} values for CaEGTA in 0.1 M KCl at 20°C and 37 °C respectively and at different pHs.

Table 1: Dissociation Constant of CaEGTA for Ca^{2+} in 0.1 M KCI*		
Kd^{EGTA} (nM)		
pH	20°C	37°C
6.50	3728	2646
6.6	2354	1672
6.7	1487	1057
6.75	1182	841
6.8	940	669
6.85	747	532
6.9	594	423
7.00	376	268
7.05	299	213
7.10	238	170
7.15	189.1	135.4
7.20	150.5	107.9
7.25	119.8	86
7.30	95.4	68.6
7.35	76.0	54.7
7.40	60.5	43.7
7.45	48.2	34.9
7.50	38.5	27.9
7.60	24.5	17.88
7.70	15.61	11.49
7.80	9.99	7.42
7.9	6.41	4.82
8.00	4.13	3.15
8.10	2.68	2.08
8.20	1.75	1.39

Directions for Use / Protocole

The buffers should be stored refrigerated to retard growth of bacterial contaminants. No preservatives (e.g., sodium azide) have been added to the solutions; therefore it is recommended that the kits be used within 3-5 months of receipt.

Protocole 1: Calibration buffer preparation

Calibrations may be performed using 2.0 mL samples in a fluorometer cuvette by a reciprocal dilution method (below) or any other method.

-Prepare a stock solution of the Ca^{2+} indicator (salt form) in any Ca^{2+}- and K+ EGTA-free buffer at approximately 100–500 times the concentration required for the measurements (typically0.2–1 mM).

-Prepare a "zero Ca^{2+} sample" and a "high Ca^{2+} sample": add to each calibration buffers (10 mM K_2EGTA; and 10 mM CaEGTA), the stock solution of Ca^{2+} indicator to give an indicator concentration of about 1–10 µM.

-Prepare reciprocal dilutions of both samples at 1 to 9mM Ca^{2+} , and record the absorption / emission spectrum of each:
. replace sequentially the volume indicated in following table starting from 2ml of the "zero Ca^{2+} sample with the same volume of the "high Ca^{2+} sample". (see table below)
. record the absorbtion / emission spectrum.
Note: avoid to illuminated solution more than require for measurment, especially with indicators that undergo excitation shifts (fura-2) or emissions shifts (indo-1) upon Ca^{2+} binding.

* Reciprocal dilutions used to prepare indicated free [Ca^{2+}].**

CaEGTA	**[Ca^{2+}]free**	**Volume to remove/replace using a 2.00 ml sample**
0.00 mM	0 µM	S0= "zero Ca^{2+} sample"
1.00 mM	0.017 µM	Replace 0.200 mL *
2.00 mM	0.038 µM	Replace 0.222 mL *

* from above with the same volume of S10
** Free Ca^{2+} Concentrations are calculated according Roger Tsien 1989 method at pH7.20 with an ionic strength of 100 mM KCl,at 20°C (the Kd of EGTA is 150.5 × 10-9 M). [Ca^{2+}]free varies with pH, temperature and ionic strength, i.e. a change in pH of 0.05 units can alter K EGTA by up to 20%. Search in the literature for Kd values.

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CaEGTA	$[Ca^{2+}]$free	Volume to remove/replace using a 2.00 ml sample
3.00 mM	0.065 µM	Replace 0.250 mL *
4.00 mM	0.100 µM	Replace 0.286 mL *
5.00 mM	0.150 µM	Replace 0.333 mL *
6.00 mM	0.225 µM	Replace 0.400 mL *
7.00 mM	0.351 µM	Replace 0.500 mL *
8.00 mM	0.602 µM	Replace 0.667 mL *
9.00 mM	1.35 µM	Replace 1.00 mL *
10.0 mM	39 µM	S10= "high Ca^{2+} sample"

- excitation or emission at a single wavelength can be plotted against $[Ca^{2+}]$free to give a calibration curve that can be used to determine the $[Ca^{2+}]$free of an unknown solution.
The fluorescence intensity and free calcium concentration has the following relationship:

$$\log\{(F-Fmin)/(Fmax-F)\} = -\log Kd + \log[Ca^{++}]$$

Plot log{(F-Fmin)/(Fmax-F)} vs. Log[Ca++]. Make sure that the unit of [Ca++] is in M (mole). The X-intercept from the linear plot is LogKd.

Other Information

For in vitro R&D use only

Please contact Uptima – Interchim for any other information

Related products

- TPEN (Tetrakis-(2-pyridylmethyl)ethylenediamine), FP-44736A
- Pluronic acid, FP-37361A
- Caged Ca^{2+}: NP-EGTA, FP-52902A
DMNP-EGTA, FP-44506A and –AM FP-M1437A
- Ionomycin, FP-53989A
- Fluo-3 AM, FP-78932A
- Fluo-8 NW, CJ2560

Literature

Roger Tsien 1989 Methods Enzymol 172, 230 (1989)
Roger Tsien 1989 Tsien, R. in Methods in Cell Biology, Vol. 30, Taylor, D.L. and Wang, Y-L, Eds., Academic Press (1989) pp. 127-156

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