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Product Information

Calcium Calibration Buffer kit (2 x 50 mL)

Catalog Number: 59100

Packaging Size: 2 x 50 mL

Component Information:
Component A (zero calcium buffer):
10 mM K_2EGTA, 100 mM KCl and 10 mM MOPS; pH 7.20
Component B (high calcium buffer):
10 mM CaEGTA, 100 mM KCl and 10 mM MOPS; pH 7.20

Color and Form: Colorless liquids

Storage and Handling
Store at 4 °C for up to three months upon receipt. Store at -20°C for up to two years upon receipt.

Product Description
The dissociation constant (K_d) of a fluorescent calcium indicator is a function of temperature, ionic strength and pH. To make accurate calcium measurements, it is important to determine the dissociation constant under a given set of conditions. The calcium calibration buffer kit is specifically designed for easy calibration of calcium indicators by providing known free calcium concentrations ranging from zero up to about 40 uM.

The kit contains two components. Component A (zero calcium buffer) is a solution comprising of 10 mM K_2EGTA, 100 mM KCl and 10 mM MOPS at pH 7.20, 20°C. Component B (high calcium buffer) is a solution comprising 10 mM CaEGTA, 100 mM KCl and 10 mM MOPS at pH 7.20, 20°C. A calcium buffer of a desired free calcium concentration between zero and 40 mM can be obtained by mixing the two components at an appropriate ratio. The free calcium concentration can be estimated using the equation:

$$[Ca^{++}]_{free} = K_d^{EGTA} \times (c_{CaEGTA}/c_{K2EGTA})$$

where K_d^{EGTA} is the dissociation constant of CaEGTA at a given temperature, ionic strength and pH; c_{CaEGTA} and c_{K2EGTA} are the starting concentrations of CaEGTA and K_2EGTA, respectively. For your convenience, Table 1 below lists K_d^{EGTA} values for CaEGTA in 0.1 M KCl at 20°C and 37 °C, respectively, and at various pH.

Traditionally, the high calcium calibration buffer is prepared using pH to monitor the complexation of calcium and EGTA to obtain a 1:1 ratio. However, this method is difficult to perform reproducibly, leading to variability in the free calcium concentration from batch to batch of high calcium buffer. To address this issue, we have implemented new quality control measures that include both pH measurement and calcium electrode measurement to ensure that the free calcium concentration in the high calcium buffer is accurate.

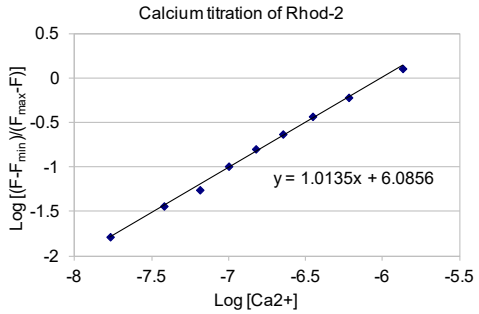

Figure 1. Example of a calcium calibration curve for Rhod-2 (Kd = 1 uM).

Protocol

A reciprocal dilution method can be used to make a series of calcium buffers. The protocol below describes how to generate 11 calcium buffers (2 mL each) with free calcium concentrations ranging from zero to 39.8 uM (Table 2 lists the total CaEGTA concentration, free calcium concentration and volume to remove/replace for each dilution).

1. Prepare a stock solution of the calcium indicator (water-soluble salt form) in water or any dilute buffer (free of calcium and any chelator) at approximately 100-500 times the concentration required for the experiment (e.g., 0.2~1 mM).
2. A small amount of the indicator stock solution is added to 2.00 mL of component A solution (zero calcium buffer) (10 mM K_2EGTA) in a 2 mL cuvette so that the final indicator concentration is in the range of 1-10 uM. This is the "zero Ca^{++}" buffer. Six mL of "high calcium buffer" is made by adding exactly three times as much calcium indicator stock solution into 6 mL of Component B solution (10 mM CaEGTA). Note that a greater amount of the "high calcium buffer" will be needed for the experiment. Make sure that the pH for both solutions are the same.
3. The appropriate spectrum (excitation or emission) of the indicator is recorded with the 2 mL zero calcium solution. After the spectrum measurement, this zero calcium solution is used for making the next solution.
4. Remove 0.20 mL of the above zero calcium solution and replace this with 0.20 mL of the "high calcium solution". The resulting solution contains 1.00 mM of total CaEGTA and 0.017 uM $[Ca^{++}]_{free}$ (See Table 2).
5. Record the spectrum again. Then remove 0.22 mL from the above 1.00 mM CaEGTA solution and replace it with 0.22 mL of the "high calcium solution". The resulting solution contains 2.00 mM total CaEGTA and 0.038 uM $[Ca^{++}]_{free}$ (See Table 2).
6. Record the spectrum. The remaining 8 solutions are prepared similarly using the "volume to remove/replace" indicated in Table 2 for each dilution. Record the fluorscence spectra for each solution.
7. The fluorescence intensity and free calcium concentration has the following relationship:
$$\log\{(F-F_{min})/(F_{max}-F)\} = -\log K_d + \text{Log}[Ca^{++}]$$
8. Plot $\log\{(F-F_{min})/(F_{max}-F)\}$ vs. $\text{Log}[Ca^{++}]$. Make sure that the unit of $[Ca^{++}]$ is in M. The X-intercept from the linear plot is $\text{Log}K_d$ (M). See Figure 1 for an example.

For more detailed information on calcium indicator calibration, please refer to the references listed below.

References:
1. Physiological Rev 79, 1089 (1999);
2. Meth Cell Biol 40, 155 (1994);
3. Meth Cell Biol 40, 3 (1994);
4. Cell Calcium 12, 279 (1991);
5. Meth Enzymol 192, 38 (1990);
6. Cell Calcium 11, 85 (1990);
7. Cell Calcium 11, 63 (1990).
8. Meth Enzymol 172, 230 (1989);
9. J Biol Chem 260, 3440 (1985).

Table 1. Dissociation Constant of CaEGTA in 0.1 M KCl*

K_d^{EGTA}(nM)		
pH	**20°C**	**37°C**
6.50	3728	2646
6.60	2354	1672
6.70	1487	1057
6.75	1182	841
6.80	940	669
6.85	747	532
6.90	594	423
6.95	472	337
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
7.90	6.41	4.82
8.00	4.13	3.15
8.10	2.68	2.08
8.20	1.75	1.39

*Data from reference 8.

Table 2. Calcium buffers prepared by reciprocal dilution method

Total CaEGTA (mM)	$[Ca^{++}]_{free}$ (uM)*	Volume to remove/replace (mL)
0.00	0.000	"zero Ca^{++} sample"
1.00	0.017	0.200
2.00	0.038	0.222
3.00	0.065	0.250
4.00	0.100	0.286
5.00	0.150	0.333
6.00	0.225	0.400
7.00	0.351	0.500
8.00	0.602	0.677
9.00	1.350	1.00
10.00	39.800	"high Ca^{++} sample"

*At 20°C

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