# ab171868

# Cal-520, AM

## Instructions for Use

For detecting calcium in cells using a proprietary fluorescence probe

View kit datasheet: www.abcam.com/ab171868

(use www.abcam.cn/ab171868 for China, or www.abcam.co.jp/ab171868 for Japan)

This product is for research use only and is not intended for diagnostic use.

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# 1. Introduction

Cal-520, AM (ab171868) provides a robust homogeneous fluorescence-based assay tool for detecting intracellular calcium mobilization. Cal-520 AM is a new fluorogenic calcium-sensitive dye with a significantly improved signal to noise ratio and intracellular retention compared to the existing green calcium indicators (such as Fluo-3 AM and Fluo-4 AM). Cells expressing a GPCR or calcium channel of interest that signals through calcium can be preloaded with Cal-520, AM which can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Cal 520, AM are cleaved by esterases, resulting in a negatively charged fluorescent dye that stays inside cells. Its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which significantly increase the fluorescence of Cal-520, AM. The characteristics of its long wavelength, high sensitivity, and >100 times fluorescence enhancement, make Cal-520, AM an ideal indicator for the measurement of cellular calcium. The high S/N ratio and better intracellular retention make the Cal-520, AM calcium assay a robust tool for evaluating GPCR and calcium channel targets as well as for screening their agonists and antagonists.

**Features**

**Organic anion transporter resistance**: Enable $Ca2^+$ assays with probenecid-interference GPCRs and $Ca2^+$ channels.

**Robust:** Significantly higher S/N ratio than those of Fluo-4 AM and any other commercially available fluorescent $Ca2^+$ assays.

**Convenient:** maximum excitation 492 nm; maximum emission 514 nm.

## 2. Kit Contents

| Components | Amount |
|---|---|
| Cal-520, AM | 1 mg |

## 3. Storage and Handling

Keep at -20°C. Protect from light.

## 4. Additional Materials Required

- A 96 or 384-well microplate: Tissue culture microplate with black wall and clear bottom.
- A fluorescence microplate reader with a filter set of Ex/Em = 490/525 nm.
- HHBS (1X Hank's with 20 mM Hepes Buffer, pH 7.0).
- 100% DMSO anhydrous.
- Pluronic F127
- Anion transporter inhibitor (optional)

## 5. Assay Protocol

This protocol for loading Cal-520, AM esters into live cells. This protocol only provides a guideline, and should be modified according to your specific needs. please read the whole protocol before starting

1. Prepare a 2 to 5 mM stock solution of Cal-520, AM esters in high-quality, anhydrous DMSO.

2. On the day of the experiment, either dissolve Cal-520 in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a Cal-520 dye working solution of 5 to 10 μM in Hanks and Hepes buffer

(HHBS) or the buffer of your choice with 0.02% Pluronic® F-127. The exact concentration of the indicator required for cell loading must be determined empirically.

*Note: The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of Cal-520, AM esters*.

3. If your cells (such as CHO cells) contain the organic anion-transports, probenecid (0.5–1 mM) may be added to the cell medium to reduce leakage of the de-esterified indicators

4. Incubate the dye-loading plate in a cell incubator for 60 to 90 minutes, and then incubate the plate at room temperature for a further 30 minutes.

   *Note: Incubation with the dye for longer than 2 hours gives a better signal intensity for some cell lines*.

5. Replace the Cal-520, AM dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove excess probes.

6. Run the experiments at Ex/Em = 490/525 nm at room temperature.

# 6. Data Analysis

To determine either the free calcium concentration of a solution or the Kd of a single-wavelength calcium indicator, the following equation is used:

$$[Ca]_{free} = K_d[F - F_{min}]/F_{max} - F]$$

Where F is the fluorescence of the indicator at experimental calcium levels, $F_{min}$ is the fluorescence in the absence of calcium and $F_{max}$ is the fluorescence of the calcium-saturated probe.

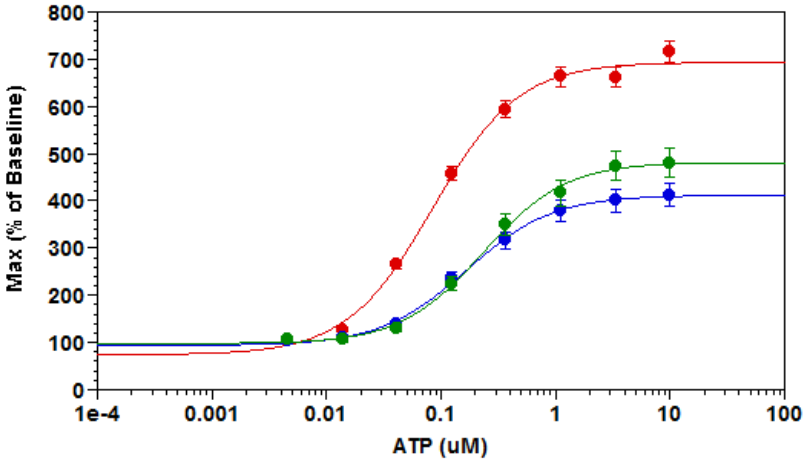


**Figure 1.** ***ATP dose response comparison in CHO-K1 cells measured with Cal-520, AM No Wash, FLIPR Calcium 4 or Fluo-4 Direct Calcium Assay Kit.*** *CHO-K1cells were seeded overnight in 60,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µL of the Cal-520, AM No Wash Calcium Assay Kit(Red), FLIPR Calcium 4 Kit(Green) or Fluo-4 Direct Calcium Assay Kit(Blue) for 1.5 hours at 37°C. ATP (50µL/well) was added to achieve the final indicated concentrations.*

# 7. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| Assay not working | Assay buffer at wrong temperature | Assay buffer must not be chilled - needs to be at RT |
| | Protocol step missed | Re-read and follow the protocol exactly |
| | Plate read at incorrect wavelength | Ensure you are using appropriate reader and filter settings (refer to datasheet) |
| | Unsuitable microtiter plate for assay | Fluorescence: Black plates (clear bottoms);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>If critical, datasheet will indicate whether to use flat- or U-shaped wells |
| Unexpected results | Measured at wrong wavelength | Use appropriate reader and filter settings described in datasheet |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Unsuitable sample type | Use recommended samples types as listed on the datasheet |
| | Sample readings are outside linear range | Concentrate/ dilute samples to be in linear range |

| | | |
|---|---|---|
| Samples with inconsistent readings | Unsuitable sample type | Refer to datasheet for details about incompatible samples |
| | Samples prepared in the wrong buffer | Use the assay buffer provided (or refer to datasheet for instructions) |
| | Samples not deproteinized (if indicated on datasheet) | Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)*** |
| | Cell/ tissue samples not sufficiently homogenized | Increase sonication time/ number of strokes with the Dounce homogenizer |
| | Too many freeze-thaw cycles | Aliquot samples to reduce the number of freeze-thaw cycles |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Samples are too old or incorrectly stored | Use freshly made samples and store at recommended temperature until use |
| Lower/ Higher readings in samples and standards | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Out-of-date kit or incorrectly stored reagents | Always check expiry date and store kit components as recommended on the datasheet |
| | Reagents sitting for extended periods on ice | Try to prepare a fresh reaction mix prior to each use |
| | Incorrect incubation time/ temperature | Refer to datasheet for recommended incubation time and/ or temperature |
| | Incorrect amounts used | Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume) |

| Problem | Reason | Solution |
|---|---|---|
| Standard curve is not linear | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Pipetting errors when setting up the standard curve | Try not to pipette too small volumes |
| | Incorrect pipetting when preparing the reaction mix | Always prepare a master mix |
| | Air bubbles in wells | Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates |
| | Concentration of standard stock incorrect | Recheck datasheet for recommended concentrations of standard stocks |
| | Errors in standard curve calculations | Refer to datasheet and re-check the calculations |
| | Use of other reagents than those provided with the kit | Use fresh components from the same kit |

## Technical Support



For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)