# ab112129 Calcium Flux Assay Kit (Fluo-8, No Wash)

For detecting calcium in cells by using our proprietary fluorescence probe
This product is for research use only and is not intended for diagnostic use.

## Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

## Materials Supplied

| Item | Amount (1 plate) | Amount (10 plates) |
|---|---|---|
| Component A: Fluo-8 | 1 vial | 1 vial |
| Component B: 10X Pluronic® F127 Plus | 1 mL | 10 mL |
| Component C: HHBS | 9 mL | 100 mL |

## Materials Required, Not Supplied

- 96 or 384-well microplate: Tissue culture microplate with black wall and clear bottom.
- HTS fluorescence microplate reader with a filter set of Ex/Em = 490/525 nm. We recommend the following instruments: FLIPR™, FDSS, BMG NOVOstar™, FlexStation, ViewLux, IN Cell Analyzer or Arrayscan.
- HHBS (1X Hank's with 20 mM Hepes Buffer, pH 7.0).
- 100% DMSO
- (Optional) Carbachol (ab141354) or other GPCR agonists to induce agonist-mediated calcium release in cells.

## Assay Procedure

- *Warning: Do not add additional probenecid.*

### Prepare Cells

1. For adherent cells: Plate cells overnight in growth medium with 5-10% FBS at 40,000 to 80,000 cells/well/100 µL for a 96-well plate or 10,000 to 20,000 cells/well/25 µL for a 384-well plate.
2. For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in HHBS at 125,000 to 250,000 cells/well/100 µL for a 96-well poly-D-lysine plate or 30,000 to 60,000 cells/well/25 µL for a 384-well poly-D-lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.

   ∆ ***Note****: Each cell line should be evaluated on an individual basis to determine the optimal cell density for the intracellular calcium mobilization.*

### Preparation of Fluo-8 dye-loading solution

1. Thaw all the kit components at room temperature before use.
2. Make Fluo-8 stock solution: Add 20 µL of DMSO into Fluo-8 (Component A) and mix them well.

   ∆ **Note:** 20 µL of Fluo-8 stock solution is enough for one plate. Unused Fluo-8 stock solution can be aliquoted and stored at -20°C for more than one month if the tubes are sealed tightly. Keep protected from light and avoid repeated freeze-thaw cycles.

3. Make 1X assay buffer: Add 9 mL of HHBS (Component C) into 1 mL of 10X Pluronic® F127 Plus (Component B) and mix them well.

   ∆ **Note**: 10 mL of 1X assay buffer is suitable for one plate. Aliquot and store unused 1X assay buffer at -20°C. Keep from light and avoid repeated freeze-thaw cycles.
4. Make Fluo-8 dye-loading solution for one cell plate: Add 20 µL of Fluo-8 stock solution (from Step B.2) into 10 mL of 1X assay buffer (from Step B.3) and mix them well. This working solution is stable for at least 2 hours at room temperature.

### Run Calcium Assay

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Fluo-8 dye-loading solution (from Step B.4) into the cell plate.

   ∆ **Note**: Alternatively, grow the cells in growth medium with 5 - 10% FBS to improve cell growth. In this case, it is important to replace the growth medium with HHBS buffer in order to minimize background fluorescence, and compound interference with serum.
2. Incubate the dye-loading plate in a cell incubator for 30 minutes, and then incubate the plate at room temperature for another 30 minutes.

   ∆ **Note**: If the assay requires 37°C, perform the experiment immediately without further room temperature incubation. If the cells can function well at room temperature for longer time, incubate the cell plate at room temperature for 1-2 hours.
3. Prepare the compound plates with HHBS or the desired buffer.
4. Using your instrument that contains a pipettor (such as Flexstation from Molecular Devices), dispense compounds directly onto to the cell plate while collecting the data simultaneously (see next step for Ex/Em instructions).

   ∆ **Note**: Typically the instrument collects the data every second for 100 seconds. Use the max signal to generate the plot. You can use a fluorescence microscope by adding the stimuli while taking the picture simultaneously.
5. Run the calcium flux assay by monitoring the fluorescence intensity at Ex/Em = 490/525 nm.

   ∆ **Note**: *It is important to run the signal test before the experiment. Different instruments have their own range. Adjust the signal test intensity to the level of 10% to 15% of the maximum instrument intensity counts.*

## Data Analysis

The reading (RFU (Max-Min)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Carbachol dose samples.

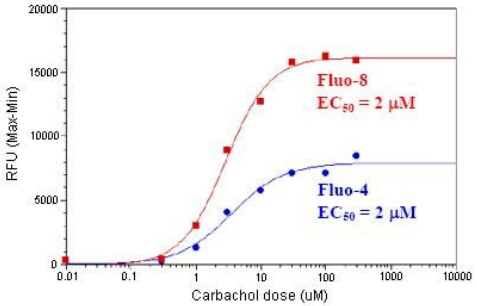


**Figure 1.** Carbachol Dose Response was measured in HEK-293 cells with ab112129. HEK-293 cells were seeded overnight at 40,000 cells/100 µL/well in black wall/clear bottom 96-well plate. The cells were incubated with 100 µL of dye-loading solution using ab112129 for 1 hour at room temperature. Carbachol (50 µL/well) was added to achieve the final indicated concentrations.

## 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 sample 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 appropriate deproteinization protocol |
| | 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) |
| 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)