

## Screen Quest™ Fura-2 NW Calcium Assay Kit

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 36320 (10 plates), 36321 (100 plates)	Keep in freezer Avoid exposure to light	FLIPR, FDSS, NOVOSTar, FlexStation

### Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). Screen Quest™ Ratiometric Calcium Assay Kit provides a homogeneous fluorescence-based assay for detecting the intracellular calcium mobilization. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with Fura-2 AM which can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Fura-2 AM are cleaved by non-specific cell esterases, resulting in a negatively charged fluorescent dye which stays inside the cells. The 340/380 nm excitation ratio allows accurate measurements of the intracellular Ca<sup>2+</sup> concentration. The characteristics of its ratio measurements reduce the effects of uneven dye loading and cell numbers, dye leakage and photo bleaching. This Screen Quest™ Ratiometric Calcium Assay Kits provide an optimized assay method with no washing step for monitoring the G-protein-coupled receptors and calcium channels. The assay can be performed in a convenient 96-well or 384-well microtiter plate format and easily adapted to automation. The kits come with all the essential components with an optimized protocol to use with FlexStation or an equivalent instrument.

### Kit Components

Components	Amount	
	Cat. # 36320 (10 plates)	Cat. # 36321 (100 plates)
Component A: Fura-2 AM	1 vial, lyophilized	10 vials, lyophilized
Component B: 10X Pluronic F127 Plus	10 bottles (1 mL/bottle)	10 bottles (10 mL/bottle)
Component C: HHBS (Hanks' buffer with 20 mM Hepes)	1 bottle (100 mL)	Not included

### Assay Protocol for one plate

#### Brief Summary

**Prepare cells in growth medium → Add Fura-2 AM dye-loading solution (100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate) → Incubate at RT for 1 hour  
→ Monitor fluorescence increase at Ex/Em = 340/510 nm and 380/510 nm**

*Caution: No additional probenecid is needed.*

#### 1. Prepare Cells:

- 1.1 For adherent cells: Plate cells overnight in growth medium 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.
- 1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in equal amount of HHBS and Fura-2 AM dye-loading solution (see steps 2.4 below) 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.*

#### 2. Prepare Fura-2 AM dye-loading solution:

- 2.1 Thaw 1 vial of Fura-2 AM (Component A), 1 bottle of 10X Pluronic F127 Plus (Component B) and HHBS (Component C) at room temperature before use.
- 2.2 Make Fura-2 AM stock solution: Add 200 µL of DMSO into the vial of Fura-2 AM (Component A), and mix them well.  
*Note: 20 µL of reconstituted Fura-2 AM stock solution is enough for one plate. Unused reconstituted Fura-2 AM stock solution can be aliquoted and stored at ≤ -20 °C for up to three months if the tubes are sealed tightly and kept from light. Avoid repeated freeze-thaw cycles.*
- 2.3 Make 1X assay buffer:

a). For **Cat # 36320 (10 plates kit)**, make 1X assay buffer by adding **9 mL** of HHBS (Component C) into the bottle of 10X Pluronic F127 Plus (Component B, 1 mL), and mix them well.

b). For **Cat # 36321 (100 plates kit)**, make 1X assay buffer by adding the whole bottle of 10 X Pluronic F127 Plus (Component B, 10 mL) into **90 mL** of HHBS buffer (not included in the kit), and mix them well.

*Note: 10 mL of 1X assay buffer is enough for 1 plate. Aliquot and store un-used 1X assay buffer at  $\leq -20^{\circ}\text{C}$  and kept from light. Avoid repeated freeze-thaw cycles.*

- 2.4 **Make Fura-2 AM dye-loading solution for one cell plate:** Add 20  $\mu\text{L}$  of DMSO reconstituted Fura-2 AM stock solution (from Step 2.2) into 10 mL of 1X assay buffer (from Step 2.3), and mix them well. This dye-loading solution is stable for at least 2 hours at room temperature.

### 3. Run Calcium Assay:

- 3.1 Add 100  $\mu\text{L}$ /well (96-well plate) or 25  $\mu\text{L}$ /well (384-well plate) of Fura-2 AM dye-loading solution into the cell plate.

*Note: If your compounds interfere with the serum, it is important to replace the growth medium with HHBS buffer (100  $\mu\text{L}$ /well for a 96-well plate or 25  $\mu\text{L}$ /well for a 384-well plate before dye-loading).*

- 3.2 Incubate the dye-loading plate at cell incubator for 1 hour, and then incubate the plate at room temperature for another 20 minutes.

*Note 1: If assays require  $37^{\circ}\text{C}$ , perform the experiment immediately without further room temperature incubation.*

- 3.3 Prepare the compound plate by using HHBS or your desired buffer.

- 3.4 Run the calcium flux assay by monitoring the fluorescence increase at Ex/Em = 340/510 nm and 380/510 nm as described in the instrument manuals.

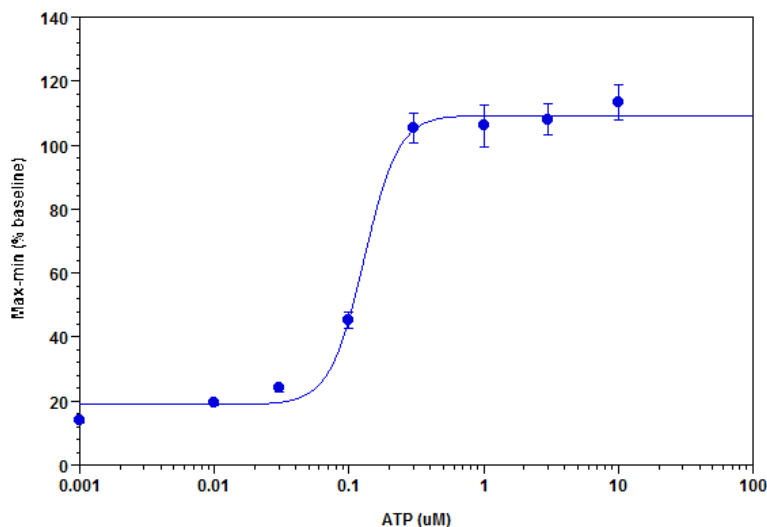
For assays performed on a FlexStation, use the following wavelength parameters:

Excitation	Emission	Cutoff
Lm1 340	510 nm	455 nm
Lm2 380	510 nm	455 nm

For assays performed on an FDSS, use the standard filters for Fura-2 calcium assays on the instrument.

*Note. Dispense speed and height for compound additions need to be optimized for each assay.*

## Data Analysis



**Figure 1.** ATP Dose Response in CHO cells measured with Screen Quest™ Ratiometric Fura-2 NW Calcium Assay Kit. CHO-K1 cells were seeded overnight at 40,000 cells/100  $\mu\text{L}$ /well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100  $\mu\text{L}$  of the Screen Quest™ Ratiometric Fura-2 NW Calcium Assay Kit for 1 hour at room temperature. ATP (50  $\mu\text{L}$ /well) was added by a FlexStation (Molecular Devices) to achieve the final indicated concentrations.

**Warning: This kit is only sold for the end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call 408-733-1055 or e-mail us at [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.**