Maximizing cell wash performance with the AquaMax® 2000/4000 microplate washers

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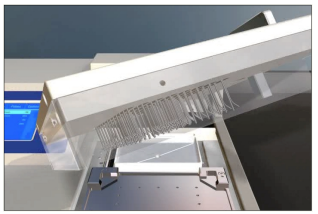
Introduction

Many cell-based applications, including calcium flux assays, cell-based ELISAs, ion channel assays, and others require gentle but thorough washing of cells in order to ensure cell retention, cell integrity and optimal assay performance. The 96 cell wash head for AquaMax® 2000/4000 microplate washers from Molecular Devices uses angled pins to dispense fluid without disturbing cells. Adjustable dispense rate and aspiration height allow users to minimize disruption of even weakly adherent cells. We demonstrate >95% retention of weakly adherent HEK293 cells on a standard tissue culture microplate surface after washing with the AquaMax microplate washer and cell wash head. We also show excellent performance of washed CHO-M1 cells in a Fluo-4 calcium flux assay run on the FlexStation® 3 multimode microplate reader.

96 cell wash head with angled dispense pins



Materials

- HEK293 cells
- CHO-M1 cells: CHO cells stably transfected with M1 muscarinic receptor
- MEM + 10% FBS + 1% pen/strep
- Ham's F12 + 10% FBS + pen/strep + 50 ug/mL G418
- 1X Dulbecco's PBS with calcium and magnesium
- Calcium wash buffer: HBSS/20 mM HEPES/2.5 mM probenecid (Sigma cat. #8761)
- Black-wall, clear-bottom 96-well tissue culture microplates (Corning cat. #3904)
- Calcein AM (Invitrogen™ cat. #C3100MP)
- Fluo-4 AM (Invitrogen™ cat. #F23917
- Carbachol (Sigma cat. #C4382)
- AquaMax® 4000 microplate washer with 96 cell wash head
- IsoCyte™ laser scanning cytometer (Molecular Devices)
- FlexStation® 3 multimode microplate reader with integrated fluidics (Molecular Devices)

Methods: Cell retention

Calcein AM labeling:
- HEK293 cells were plated at 10,000 cells per well in 96-well non-coated tissue culture-treated microplates.
- Calcein AM was added to wells for a final concentration of 0.5 uM, and cells were incubated at 37°C for 30 minutes.

Wash:
- Excess calcein AM dye was removed with a gentle pre-wash prior to test washes.
- Test washes were performed using the following settings:
  - ➢Aspirate Center; Rate = 5; Descent speed = Fast; Dwell time = 2.0 s; Probe height = 3.0 mm
  - ➢Dispense Rate = 1 or 2; Volume = 300 µL
  - ➢Aspirate: Same settings as step 1
  - ➢Repeat 2x from step 2.

Measurement of cell retention:
Plates were scanned on an IsoCyte laser scanning cytometer to obtain values for percentage of the well area covered by cells in each well of the test plates.

Results: Cell retention

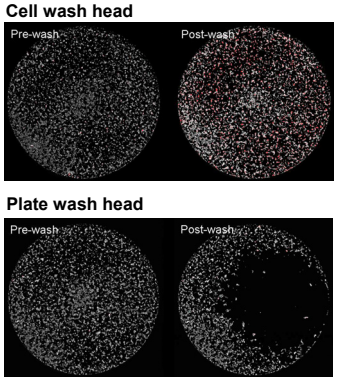


Figure 1. Images of HEK293 cells washed with cell wash head (angled dispense pins; top row) or plate wash head (straight dispense pins, bottom row). Cell retention is >95% with the cell wash head, but only 44% with the plate wash head.

Percent cell retention using different aspirate heights and dispense rates				
Replicate	Aspirate Height 2 mm	Aspirate Height 2.5 mm	Aspirate Height 3.0 mm Dispense rate 1	Aspirate Height 3.0 mm Dispense rate 2
Plate 1	87.6	94.5	96.2	98.0
Plate 2	80.8	88.5	98.6	100
Plate 3	83.7	86.2	95.7	97.1

Table 1. Percent cell retention for different aspirate heights and dispense rates tested. Three plates were analyzed per test condition. Using an aspirate height of 3.0 mm yielded the best cell retention.

Methods: Fluo-4 calcium assays

Cell culture and dye loading:
- CHO-M1 cells were plated at 50,000 cells/well the day prior to assay.
- Cells were loaded with 2 uM Fluo-4 for one hour.

Cell washing:
- Cells were washed three times with calcium wash buffer using an AquaMax® 4000 microplate washer with cell wash head and the following settings:
  - ➢Aspirate Center; Rate = 5; Descent speed = Fast; Dwell time = 2.0 s; Probe height = 1.0, 2.0, or 3.0 mm
  - ➢Dispense Rate = 1 or 2; Volume = 300 µL
  - ➢Aspirate: Same settings as step 1
  - ➢Repeat 1x from step 2.
  - ➢Final aspiration at 4.5 mm probe height to leave 100 uL of wash buffer
- Some cells were wash manually for comparison.

Calcium flux assays
- Calcium flux was measured with a FlexStation® 3 microplate reader using the Flex (fast kinetic) read type.
- Cells were stimulated with concentrations of carbachol (agonist) ranging from 10 uM to 0.5 nM. Agonist was delivered to assay wells using the FlexStation 3 system's integrated pipettor.
- All data were collected and analyzed using SoftMax® Pro software.

Results: Calcium assays

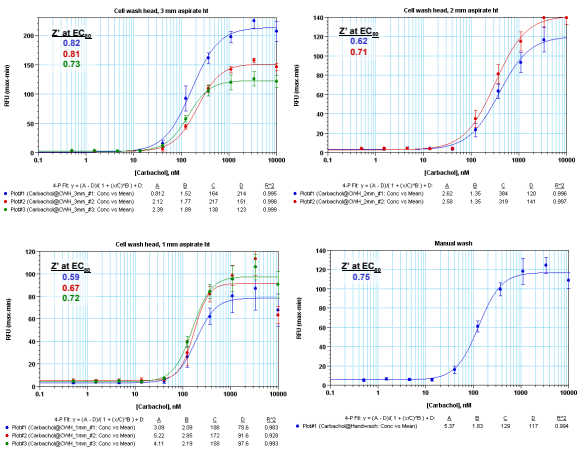


Figure 2. Fluo-4 assays run on a FlexStation® 3 plate reader: carbachol concentration response curves for CHO-M1 cells washed using the AquaMax® 4000 microplate washer with cell wash head, or manually washed. Using an aspirate height of 3 mm generally yielded the best assay results, comparable to those obtained with careful manual washing of the cells. Variability in assay window within the same wash condition is likely due to different cell passages used and slight day-to-day variation in cell plating and dye loading.

Conclusion

- The AquaMax® 2000/4000 microplate washers with optimized cell wash head provide users with optimized conditions to wash cells for demanding cell-based assays.
- Angled dispense pins deliver wash solution without disturbing even weakly adherent cells.
- Cell wash heads are compatible with existing AquaMax microplate washers.
- Cell loss is reduced to less than 5% by optimizing wash conditions for the cell type used. Washed cells perform well in Fluo-4 calcium assays, with Z' factors of 0.7 and higher.

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