Suspension Cell FACE™

Catalog No: 48305

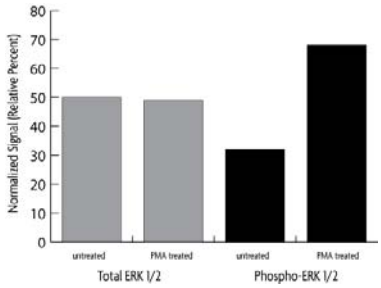
Background:
Suspension Cell FACE™ has been validated with FACE™ ERK1/2, JNK and p38 Kits using Jurkat cells and is formatted for use with all FACE™ Kits when using a suspension cell line.

Wash steps are performed by applying a vacuum, with an appropriate manifold, to the filter plate in order to remove reagents rather then inverting the plate over the sink. Failure to use vacuum for reagent removal will lead to cell loss and inconsistent results.

You must use the Suspension FACE Blocking Buffer with the 96-well filter plates. DO NOT USE the Blocking Buffer provided in regular FACE Kits as it will clog the filter plate.

Suspension Cell FACE™ includes:
Two 96-well filter plates for tissue culture
25 ml Suspension FACE™ Blocking Buffer
50 µl Hoechst Stain (1 mM)

Filter plates must be used with an appropriate vacuum manifold such as Promega Vac-Man® 96 or Millipore MultiScreen Vacuum Manifold.



Preparation of Suspension Cells
1. Grow cells in 100 mm plates and use one plate per treatment condition.
2. Count cells and, when at the desired density, pellet by centrifugation (1500 rpm for 5 minutes).
3. Cells should be serum-starved or with low serum. Resuspend in media with 0 to 0.5% FBS at the desired cell density. We recommend seeding Jurkat cells at 0.2 to 1.0 x10^6 cells/ml.
4. Seed the 96-well filter plate with 100 µl of the cell suspension. Incubate at 37°C overnight (16-18 hours).

Suspension Cell FACE Protocol
1. Cells should be stimulated with the specific stimulating agent according to the protein or pathway of interest. Serum-free media should be prepared containing a 5X final concentration of treatment. If desired, 1% BSA can be added to the media instead of serum.
2. Add 25 µl of 5X treatment mix to desired wells and bring total volume up to 125 µl. Add 25 µl media minus treatment to control wells. Incubate as desired.
3. Fix cells by adding 15 µl of 37% formaldehyde to all wells and incubate at room temperature for 20 minutes. Cover plate with parafilm to avoid escape of formaldehyde vapors.

Note: Formaldehyde is highly toxic. Confine vapors to a chemical hood and wear appropriate gloves and eye protection when using this chemical.
4. Apply vacuum to remove media and formaldehyde.
5. Wash cells 3 times with 200 µl of Wash Buffer. Each wash step should be performed for 5 minutes with gentle shaking, and the washes are removed by applying the vacuum.
6. Apply vacuum to remove last Wash Buffer, add 100 µl of Quenching Buffer and incubate for 20 minutes at room temperature.
7. Apply vacuum to remove Quenching Buffer and wash cells 2 times for 5 minutes each with 200 µl of Wash Buffer.
8. Apply vacuum to remove Wash Buffer and add 100 µl of Suspension Cell FACE Blocking Buffer (3% BSA in PBS) and incubate for 60 minutes at room temperature.
9. Apply vacuum to remove Suspension Cell FACE Blocking Buffer and wash cells 2 times with 200 µl of Wash Buffer.

10. Apply vacuum to remove Wash Buffer and add 40 µl of diluted primary antibody as specified in your FACE manual to each well. Cover with parafilm or place inside a sealed bag and incubate overnight at 4°C.
11. Apply vacuum to remove primary antibody and wash cells 3 times for 5 minutes each with 200 µl of Wash Buffer.
12. Apply vacuum to remove Wash Buffer and add 100 µl of diluted secondary antibody (1:2000) and let incubate for 1 hour at room temperature. During this incubation transfer the amount of Developing Solution required for the assay into a secondary container and leave at room temperature for at least an hour (avoid light).
13. Apply vacuum to remove secondary antibody and wash cells 3 times for 5 minutes each with 200 µl of Wash Buffer and then 2 more times with 200 µl of 1X PBS.
14. Apply vacuum to remove PBS and add 100 µl of Developing Solution to each well and let sit for 2-20 minutes (Protect from light). Monitor the blue color development until the darkest-staining wells are medium- to dark-blue. Do
not overdevelop.
15. Add 100 µl of Stop Solution and read plate within 5 minutes at 450 nm with a reference wavelength of 650 nm.

OPTIONAL: Cell Number Normalization with Hoechst Stain
1. Remove media using a vacuum manifold and wash two times with Wash Buffer for 5 minutes each wash.
2. Make a 4 µM dilution of Hoechst Stain by diluting 20 µl of the provided 1 mM stock into 4.98 ml of PBS (5 ml is sufficient for one 96-well plate). Add 50 µl of PBS with 4 µM Hoechst Stain to each well and incubate at room temperature in the dark for 10 minutes. Staining incubation can go for 10 to 30 minutes if needed.
3. Vacuum filter contents of wells and replace with 200 µl Wash Buffer. Incubate for 5 minutes at room temperature and repeat wash a second time.
4. Vacuum filter wells and add 50 µl PBS per well to read fluorescence.
5. Read fluorescence using a fluorescent plate reader at a wavelength of 360 nm excitation and 460 nm emission.

Storage
Filter plates, Suspension FACE™ Blocking Buffer and Hoechst Stain are to be stored at 4°C. Reagents are guaranteed for six months after date of purchase when stored properly.