March 19, 2012

Product Information

AccuEasy™ Flow Cytometry Kit

Catalog Number: 30069

Storage: 4°C

Stability: Stable for at least 6 months from date of receipt if stored as directed.

Kit Components:

Component	Size	Number of assays (1 assay/well)
AccuEasy Cell Lift Solution	50 ml	48-well plates: 500 24-well plates: 250
AccuEasy Stabilization Solution	50 ml	

Material required but not provided:

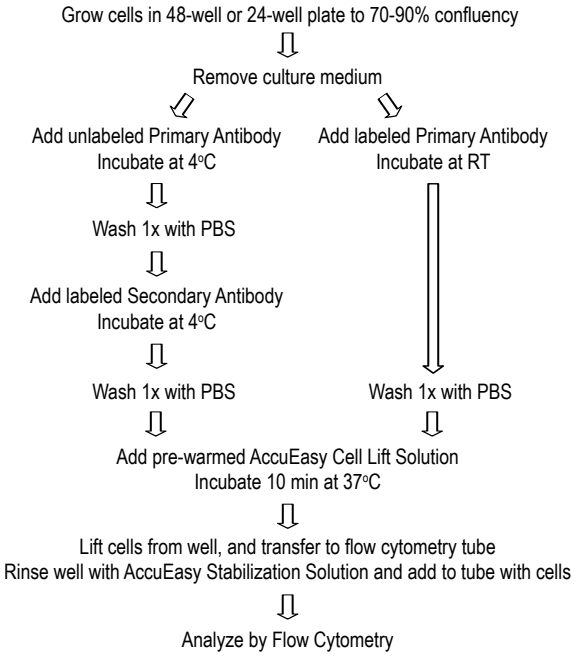
PBS
Antibodies
Antibody diluent (e.g. PBS + 1% BSA)

Product Description

The conventional method for cell surface staining of adherent cells for flow cytometry requires dissociation of cells from their culture surface prior to antibody staining. Unfortunately, cell dissociation exposes cells to significant stress and can result in rapid downregulation of cell surface markers by internalization. Additionally, the multiple centrifugation steps required during antibody staining are not only laborious, but can result in cell loss.

The AccuEasy Flow Cytometry Kit provides a new, accurate and centrifuge-free method for staining cell surface markers on adherent cells. In the AccuEasy method, antibodies are incubated with adherent cells while they remain on the culture surface. After antibody binding, the non-enzymatic AccuEasy Cell Lift Solution is used to detach the antibody-bound cells from their culture surface, followed by addition of AccuEasy Stabilization Solution to preserve cell integrity for flow cytometry analysis. This method allows you to detect cell surface marker expression on cells in their native adherent state without the perturbation caused by cell detachment. The AccuEasy method can yield substantially higher fluorescence signal for cell surface markers compared to conventional methods (Figure 1, next page).

Assay Outline



Assay Protocol

1. Seed cells onto a 24 or a 48 well plate and grow them to 75-90% confluence for the assay. Be sure to seed enough wells for samples and controls (1 assay/well). See Helpful Tips for cell seeding guidance.

2. Prepare the antibody(s) to the appropriate concentration normally used in a flow cytometry experiment. For each 48-well, 100 uL antibody solution is used. For each 24-well, 200 uL is used. Place the antibody solution on ice.

3. Take the plate out of the incubator.
   a. For unlabeled primary antibody, chill the plate on ice.
   b. For labeled primary antibody, leave the plate at room temperature.

4. Aspirate media and add 100 uL cold antibody solution into each 48 well or 200 uL into each 24 well.

5. Incubate on a rocker for the appropriate amount of time required for antibody binding. See Helpful Tips for recommendations.
   a. For unlabeled primary antibody, incubate at 4°C or on ice to avoid internalization of the receptor-antibody complex.
   b. For labeled primary antibody, incubate at room temperature. Then skip to Step 10.

6. During the primary antibody incubation, prepare the fluorescent-labeled secondary antibody conjugate at the appropriate concentration based on manufacturer's recommendation. Place on ice.

7. After incubation, aspirate the antibody solution and rinse 1X with 1 mL ice cold PBS.

8. Add 100 uL (48 well) or 200 uL (24 well) of fluorescent-labeled secondary antibody conjugate.

9. Incubate 30-60 minutes on a rocker at 4°C or on ice. During this time, warm AccuEasy Cell Lift Solution to 37°C.

10. Aspirate the labeled antibody solution and wash with 1 mL 1X PBS.

11. To each well add 100 uL (48 well) or 200 uL (24 well) AccuEasy Cell Lift Solution.

12. Incubate at 37°C for 10 minutes. Observe by microscopy to see if cells have been detached from each other. If not, continue 37°C incubation for additional 10 to 20 minutes.

13. Place the plate on ice.
    a. For cells that are not strongly adherent, dissociate cells by pipetting the cells a few times in each well and transfer to flow cytometry tube. Place the tube on ice.
    b. For cells that are strongly adherent, scrape the cells from the bottom of the plate using a Mini Cell Scraper (catalog #22003), then dissociate cells by pipetting the cells up and down and transfer flow cytometry tube. Place the tube on ice. See Helpful Tips for additional guidance on hard-to-dissociate cells.

14. Rinse the well with 100 uL AccuEasy Stabilization Solution and transfer to the corresponding flow cytometry tube on ice.

15. Analyze samples by flow cytometry within 2 hours. Alternatively, fix the samples as described in Helpful Tips (next page) and analyze the next day.

Helpful Tips:

1. Typical seeding densities for cells to be assayed on next day are $0.8x10^5$ cells per 48 well or $1.5x10^5$ cells per 24 well. Seeding density may require optimization for different cell types.

2. For cells grown in serum free media, include 10% calf serum in the diluent for primary antibody staining to block Fc receptors on the cells.

3. When staining a full plate, perform each step one row at a time to prevent the cells from drying out after aspiration.

4. Antibody binding to cells in the adherent state is as efficient as binding to cells in the suspension state. Follow your regular protocol for antibody binding.

5. When pipetting cells to break up any remaining cell clumps, be sure to pipet each well the same number of times before transferring cells to flow cytometry tubes.

6. For highly adherent cells such as A431, you may want to use a 24-well plate for higher cell number. At Step 13, after scraping cells off the wells and dissociating by pipetting, transfer cell suspension into a microcentrifuge tube and run the bottom of the tube across a row of wells of an empty Styrofoam 15 mL conical tube rack to break up cell clumps. Then transfer cells to flow cytometry tubes.

7. If you do not plan to perform flow cytometry within 2 hours of staining, fix cells in formaldehyde overnight and perform flow cytometry the next day. To fix cells in 2% formalin, add ¼ volume of 10% formalin (4% formaldehyde in PBS) to the cell suspension after step 14. Alternatively, add an equal volume of Biotium's cell fixation buffer (catalog #22015) to the cell suspension after step 14. Store overnight at 4°C, protected from light.

Related Products

Cat.#	Product Name	Unit Size
22003	Mini Cell Scrapers	pack of 200
23006	Flow Cytometry Fixation/Permeabilization Kit	50 tests
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
22017	Permeabilization and Blocking Buffer	100 mL
22010	10% Fish Gelatin Blocking Buffer	100 mL
22011	Fish Gelatin Powder	2 x 50 g
22014	30% Bovine Serum Albumin Solution	100 mL
22012	Dry Milk Powder	4 x 25 g
22002	Tween®-20	50 mL

Please visit **www.biotium.com** to view our full selection of products featuring bright and photostable fluorescent CF™ dyes, including secondary antibodies and Mix-n-Stain™ antibody labeling kits, and R-PE dye conjuages. Biotium also offers a variety of apoptosis and cell viability assays for flow cytometry analysis, including mitochondrial membrane potential dyes, fluorescent Annexin V conjugates, and NucView™488 Caspase-3 Substrate for live cells.

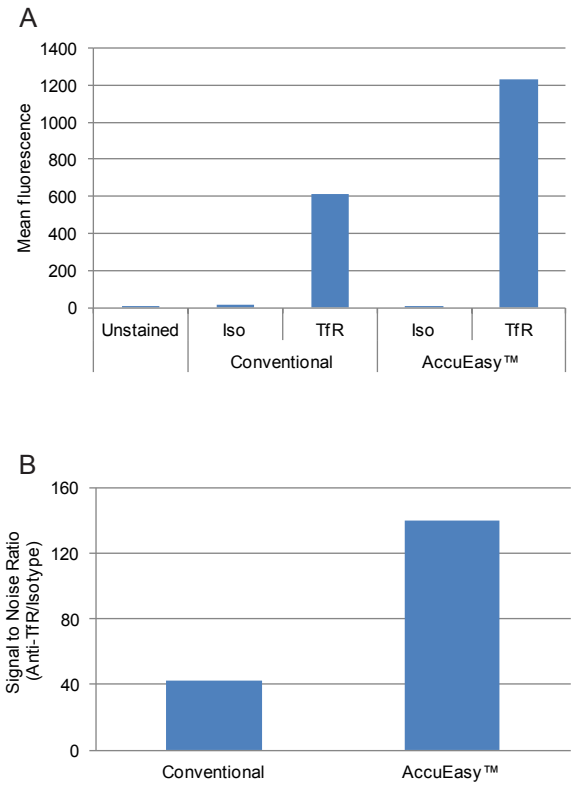


Figure 1. HeLa cells grown in a 24-well plate were stained with CF™488A-conjugated anti-transferrin receptor antibody (TfR) or CF™488A-conjugated isotype control antibody (Iso) using either a conventional staining method (cell detachment by scraping, followed by staining in suspension), or the AccuEasy protocol. Fluorescence was analyzed using a BD FACSCalibur flow cytometer in the FL1 channel. **A.** Bars represent the relative fluorescence of the geometric means of the cell populations. **B.** Bars represent the signal to noise ratio (TfR mean fluorescence/ Iso mean fluorescence). Mouse anti-TfR and isotype control antibodies (BD Biosciences) were conjugated to CF™488A using Biotium's Mix-n-Stain™ antibody labeling kits.