888.267.4436
techsupport@origene.com
www.origene.com

Product Data Sheet for CellPainter Organelle Marker Vectors - Endosome

Catalog Number: RC100025

Description: Rab4 with N-tGFP tag for Endosome marking (10ug transfection-grade plasmid). The plasmid was purified by ion-exchange column and then lyophilized.

GeneBank Accession Number: NM_004578

Cloning Vector: pCMV6-AN-GFP

Bacterial selection marker: Ampilicin (100ug/ul)

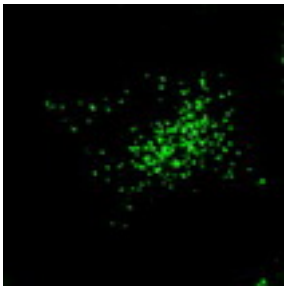
Mammalian cell selection marker: Neomycin

Amount: 10ug

Storage Condition: The product is shipped at ambient temperature. It should be stored at -20°C upon arrival.

Application: The vector can be used to transfect mammalian cells for fluorescent labeling of Endosome.
To amply the plasmid, transform the plasmid into competent DH5alpha and select with 100ug/ml ampicilin.

Validation Data:



RC100025 was transfected into SKOV3 cells. The cells were fixed 30-36 hrs later and the image was generated with a confocal microscope.

Brief Protocol:

1. Culture cells in log growth phase (about 60-70% confluence) in a 6 well-plate with sterile cover slides in wells.
2. Resuspend the CellPainter DNA in 20ul of water. Use 1-5 ug of DNA to perform transfection with the standard transfection techniques.
   When use OriGene's Turbofectin 8.0 transfection reagent, please see the protocol
3. Incubate cells for 30-36 hours in CO2 incubator. Do not culture cells longer than 48 hours.
4. Wash cells 1 x with cold PBS and then fix cells with 4% formaldehyde in PBS for 10-20 min at room temperature. Wash cells 3 x with cold PBS.
5. Carefully transfer the cover slip from well with cells face down to a glass slide with one drop of any commercial mounting medium with or without DAPI (for nuclear staining). We recommend to use VECTASHIELD® or VECTASHIELD® with DAPI mounting medium (Vector Laboratories. http://www.vectorlabs.com) which provides strong initial fluorescence and retards photobleaching over time for fluorescence microscopy. Seal cover slips with nail polish or a plastic sealant for long time storage. Mounted slides should be stored at 4 ºC. The fluorescence will remain for at lest several days.
6. View slides by confocal fluorescence microscopy within 24 - 48 hours.

Note: If immunofluorescent staining required for co-staining, follow the standard immunofluorescent staining protocol. Conventional fluorescent microscope can be used for viewing some of the organelle or subcellular structures. However the images are usually lack the desired clarity. Confocal microscope is strongly recommended for obtaining sharp organelle images. All of OriGene's validation data was obtained using a confocal microscope