TECHNICAL MANUAL

ES-Cellect™

Contents

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General information

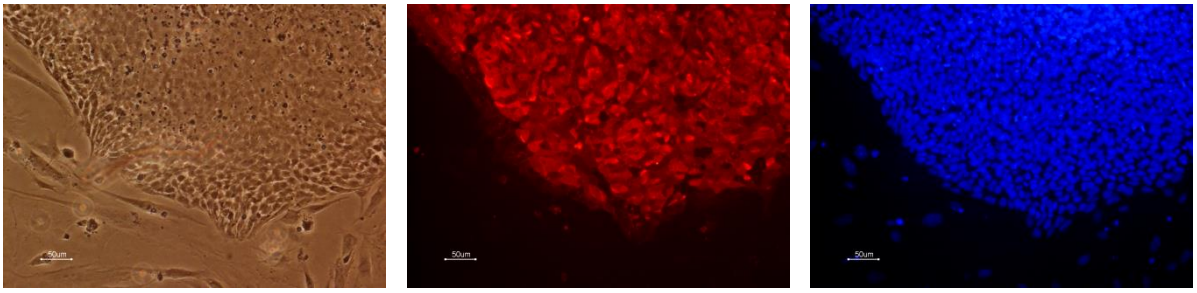
CATALOGUE #:	MAB-802-VIAL/Y20011
DESCRIPTION:	Monoclonal antibody derived from hybridoma cells produced by the fusion of mouse myeloma cells and spleen cells from BALB/C mice immunized with purified whole undifferentiated human embryonic stem (hES) cells.
FORMULATION:	150 µg of affinity purified mouse monoclonal antibody, lyophilized.
ISOTYPE:	IgG1
IMMUNOGEN:	Purified, whole cell preparation of undifferentiated hES cells.
SPECIFICITY:	This antibody has been selected for its ability to recognize a surface antigen expressed in undifferentiated hES- and mouse embryonic stem (mES) cells as well as in human induced pluripotent stem (hiPS) cells. ES-Cellect™ has been shown negative for human Foreskin Fibroblast (hFF) and mouse Embryonic Feeder (mEF) cells. See figure 1.
RECONSTITUTION:	Dissolve the antibody in 150 µl of distilled water for a 1mg/ml stock solution. The recommended concentration of the antibody is 0.5 µg/ml to 1 µg/ml; however the optimal working concentration should be determined by the user.
TESTED APPLICATIONS:	Immunocytochemistry, flow cytometry
TRANSPORTATION:	ES-Cellect™ is delivered in ambient temperature.
STABILITY & STORAGE:	The lyophilized product is stable for 12 months from the shipment date if stored at +2-8°C. The reconstituted antibody is stable for 6 weeks when stored at +2-8°C. For long-term storage it is recommended that the solution is frozen in working aliquots containing 1:1 glycerol and stored at -20 °C (frost-free freezers are not recommended). Repeated freeze thawing cycles should be avoided.
STERILITY:	ES-Cellect™ has been tested negative for bacteria and fungus infection. The hybridoma producing cells have been tested negative for mycoplasma.
USER INSTRUCTION:	Takara Bio Europe AB recommends the use of reagents according to this technical manual. Takara Bio Europe AB cannot give technical feedback on customer experiments unless the instructions in the technical manual have been followed. Takara Bio Europe AB recommends that this product is handled only by persons who have been trained in laboratory techniques and in accordance with the principles of good laboratory practices.
INTENDED USE:	ES-Cellect™ is intended for research use only and are not to be used for any other purposes including, but not limited to: unauthorized commercial purposes, *in vitro* diagnostic purposes, *ex vivo* or *in vivo* therapeutic purposes, in foods, drugs, medical devices or cosmetics of any kind, or for consumption by or use in connection with or administration or application to humans or animals.
SAFETY PRECAUTIONS:	Upon arrival check all materials for leakage or breakage.

Material and Preparation

Dulbecco Phosphate-Buffered Saline with Ca2+ and Mg2+ (D-PBS+/+)
Fixative: 4% formaldehyde solution
Blocking solution: 5% skim milk powder in D-PBS+/+, prepared and sterile filtered prior to use
Antibodies: ES-Cellect™ and anti-mouse IgG Alexa Fluor 488 or 594
Antibody dilution: 1% bovine serum albumin fraction V (BSA V) in D-PBS+/+
Mounting media: Mounting media with DAPI

Staining Protocol

1. Prepare the working solution prior to the staining procedure.
2. Wash the cells in D-PBS+/+ and fix the cells in fixative for 10 minutes at room temperature (RT).
3. Wash twice in D-PBS+/+.
4. Block in blocking solution for 30 minutes at RT.
5. Incubate cells in ES-Cellect™(dilution: 0.5-1 µg/ml) overnight at 4°C.
6. Wash 3 times in D-PBS+/+.
7. Incubate the cells in anti-mouse IgG at RT (in the dark) for one hour.
8. Wash 3 times in D-PBS+/+.
9. Mount the cells in mounting media.



[a] [b] [c]

Figure 1.
hESC cultured on mEF, stained with ES-Cellect (1 µg/ml) and goat anti-mouse IgG Alexa Fluor 594 (2 µg/ml). Staining performed according to the protocol above. Pictures are shown in 20x magnification; scale bar corresponds to 50 µm. [a] Bright field, [b] Alexa Fluor 594, [c] DAPI.

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