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Immunofluorescence (IF) staining of MTSs on MWC-chips

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MTS

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We use this protocol and it's working

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Abstract

Immunofluorescence (IF) staining of MTSs on MWC-chips (CD44,ALDH1A1)

Materials

4% paraformaldehyde (Beyotime), 1% Triton-X 100 in PBS, 10% goat serum, primary antibodies against ALDH1A1 (Alexa594-conjugated, Abcam), CD44 (Alexa488-conjugated, Abcam), FAP (Alexa647-conjugated, RD System), Hoechst 33342 dye, confocal microscopy (OLYMPUS), ImageJ software.

Immunofluorescence (IF) staining of MTSs on MWC-chips

1 Once MTSs were formed after 5 days of culture, the SC-Medium was aspirated from the culture dish, and the MTSs in microwells were fixed with 4% paraformaldehyde (Beyotime) for 20 minutes.

2 Subsequently, they were washed with PBS.

3 The MTSs were then permeabilized with 1% Triton-X 100 in PBS for 30 minutes and blocked with 10% goat serum for another 30 minutes.

4 Next, cells were incubated overnight at 4°C with primary antibodies against ALDH1A1 (Alexa594-conjugated, Abcam), CD44 (Alexa488-conjugated, Abcam), and FAP (Alexa647-conjugated, RD System).

5 Nuclei were stained using Hoechst 33342 dye.

6 Images were captured using confocal microscopy (OLYMPUS) and were analyzed using ImageJ software.