IHC on frozen sections using recombinant antibodies (2 days protocol):

Day 1

1. Wash slides 1 time **thoroughly** with cold PBS to remove cryo reagent

2. Incubate the PFA fixed samples for 15 minutes with 100mM glycine

3. Wash slides 3 times with cold PBS (change PBS between wash steps).

4. Incubate slides 15 minutes with PBST (PBS+TritonX) at room temperature.

5. Wash slides 2 times with cold PBS (change PBS between wash steps).

6. Dry edges around sample area with tissue paper and add circle with PAP-pen around each sample.

7. Incubate slides for 45 minutes with 100 µL Block Buffer (+ BSA: rb, ms / -BSA: gt).

8. Remove Block Buffer.

10. Incubate overnight with 100 µL **mix of primary antibodies** at 4°C in black incubation box.

Day 2

12. Remove **primary antibodies** and wash 4 times 15 minutes with cold PBS (change PBS between wash steps).

13. Incubate for 1h30 minutes with 100 µL **mix of secondary antibodies** at room temperature (protect from light!) in black incubation box.

14. Remove **secondary antibodies** and wash 4 times 15 minutes with cold PBS**T** (change PBS**T** between wash steps).

15. Rinse 1 time with distilled water

16. Remove water and add 1 drop of Vectashield mounting medium with DAPI.

17. Add cover glass, fix edges with nail polish and examine slides with fluorescence microscopy.

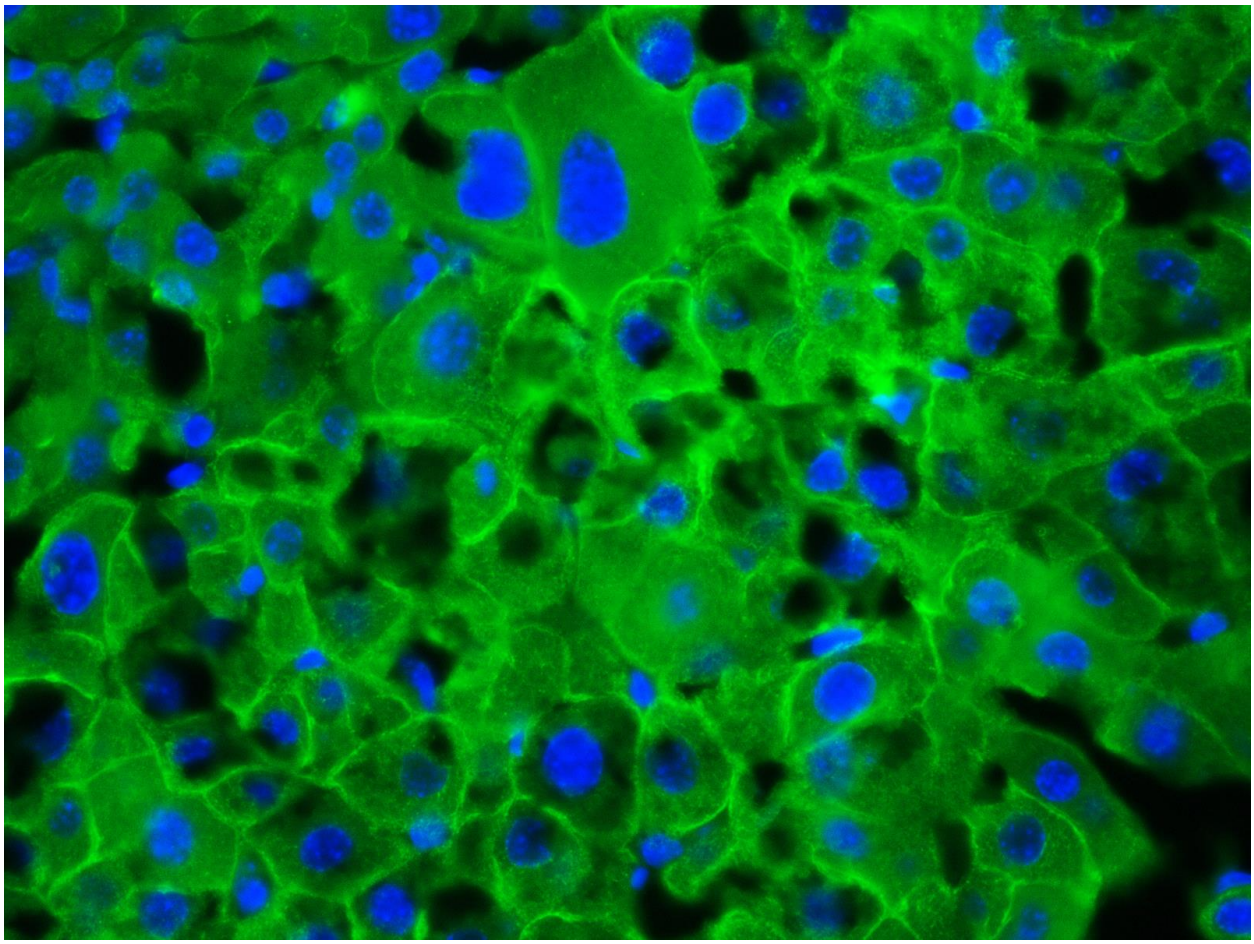

Figure: Identification of xenografted human stem cell-derived hepatocytes in frozen murine liver sections. Primary antibody: Abcam ab93741 – anti-Krt18 rabbit monoclonal antibody (dilution 1/100), secondary antibody anti-rabbit (DyLight 488 - 1/500).