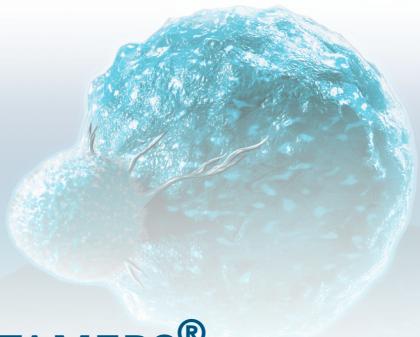
iba Solutions For Life Sciences

Short protocol



Staining of antigen-specific CD8+ T cells with MHC I Streptamers®

For staining of approx. 1 – 2 x 10^6 cells

All steps should be performed at temperatures suggested!

Cell preparation (step 1)

4° C

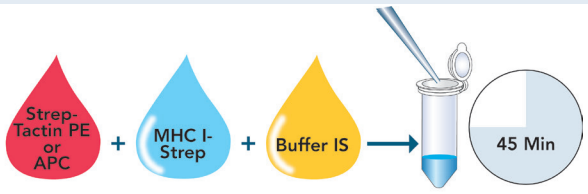
1.

Isolate PBMCs e.g. by density gradient centrifugation. Please adjust cell density to 10^7 cells / 100 µl before starting the protocol.

MHC I Streptamer® preparation (step 2)

4° C

2.

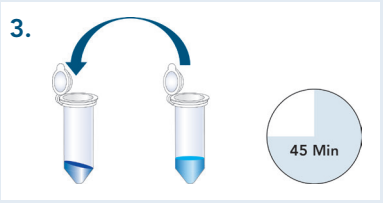


Incubate 1 µl Strep-Tactin-PE or -APC and 0.8 µl MHC I-Strep in a final volume of 10 µl Buffer IS for 45 minutes or overnight to obtain Streptamers®.

Cell labeling (steps 3 – 4)

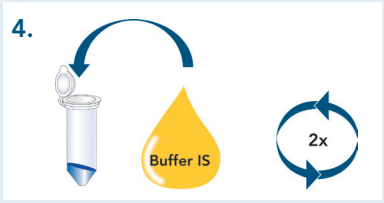
4° C

3.



Add the Streptamers® (step 2) to the cells and incubate for further 45 minutes.

4.

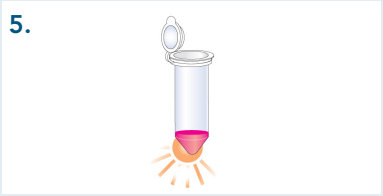


Wash cells twice with 200 µl Buffer IS.

FACS analysis or FACS sorting (step 5)

4° C

5.

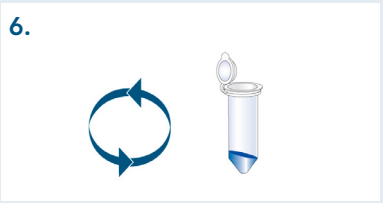


FACS analysis or optional: FACS sorting can be done. Dead cell exclusion is strongly recommended (e.g. propidium iodide, 7-AAD, etc.).

Dissociation of MHC I Streptamer® reagents (steps 6 – 8)

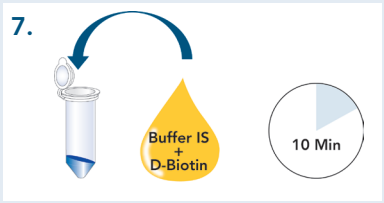
4° C

6.



Collect cells by centrifugation.

7.



Resuspend cells in 200 µl Buffer IS containing 1 mM D-Biotin and incubate for 10 min.

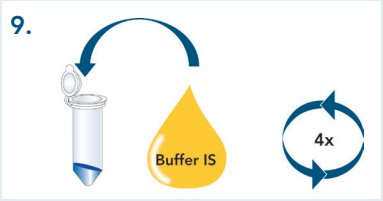
8.

Repeat steps 6 and 7.

Removal of MHC I-Streps (steps 9 – 10)

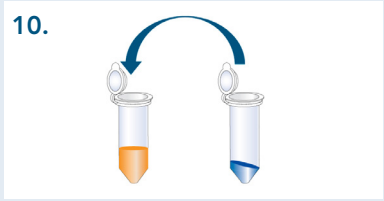
4° C

9.



Wash cells four times with 200 µl Buffer IS.

10.



Transfer cells to appropriate buffer or medium for further applications.

For detailed instructions please refer to the comprehensive MHC I Streptamer® manual at **www.iba-lifesciences.com/technical-support.html.**
Contact us for more information or technical assistance.

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