Circulating Rare Cells (CRCs) Technology

INSTRUCTION FOR USE OF ScreenCell® Molecular Biology (cat# MB 4LC)

DNA/RNA PROTOCOL FOR LIVE CRCs

DNA and RNA extraction from whole blood CRCs

ScreenCell® Live Cell (SC LC) Dilution Buffer (DB) preparation

Add B2 and C reagents to 150 ml of buffer D. Invert the bottle twice. The reconstituted buffer is sterilized by autoclaving and filtrated through a 0.22 µm Millipore filter unit. The reconstituted buffer must be stored at 4℃ and used within 12 hours.

Dilution of Blood with the Dilution buffer

a.Collect the blood sample in an EDTA tube. Invert the tube 3 times and keep at + 4℃. Blood must be filtered within 3- 4 h ours after drawing.
b. Transfer 1 ml of blood into one 15 ml RNase-/DNase- free conical tube.
c.Add 7 ml of LC DB.
d.Close the tube, homogenize once by inverting the tube and incubate 2 minutes at room temperature.

Blood Filtration

a.Remove the protective membrane at the extremity of **module B** (1).
b.Put the SC MB-LC Unit on a dry and clean place.
c.Fill **module A** with the prepared 8 ml of diluted blood (2).
d.Insert **module C** and push fully upward into **module B** until filtration starts (3).

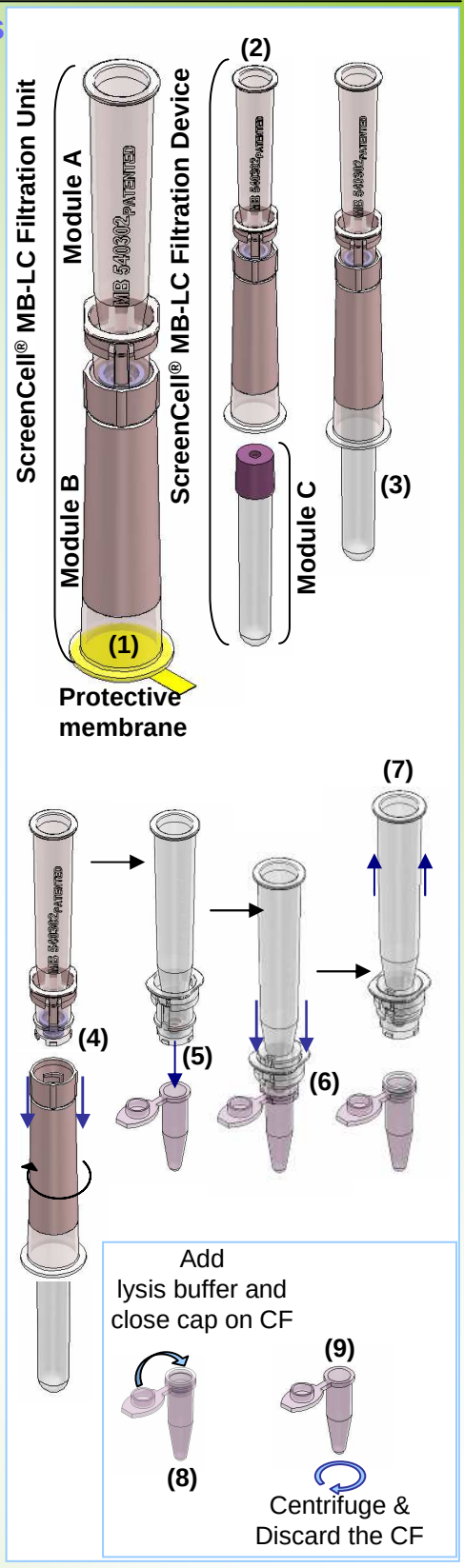
e.Before filtration is completed, add 1ml RNase-/DNase- free PBS 1X in module A and keep filtering to rinse out the filter until all liquid is gone.

Note: filtration is usually completed within approximately 50 seconds (a sample must be considered coagulated when filtration exceeds 60 seconds).

f.At the end of filtration, unscrew, remove and discard **modules B including C** from **module A** (4).
g.Hold the **module A** onto a Nuclease-free Eppendorf tube (5), release and insert the capsule-filter (CF) into the Eppendorf tube by pushing down a rod located at the bottom part of **module A** (6).
h.Once the CF has been inserted into the tube, remove and discard the rest of **module A** (7).

Cell lysis for DNA/RNA extraction

a.In each CF, add a volume** of lysis Buffer***. Close the CF with the Eppendorf tube cap (8).
b.Incubate at appropriate temperature***.
c.Centrifuge the Eppendorf tube/CF 1 min at 12 000 g (10 500 rpm) (9).
d.Discard the CF, save and store the flow-through in the closed Eppendorf tube with the cap lock.



***1.5 ml Eppendorf tube PCR Clean, cat# 3810X.**

****210 µL for DNA or 165 µL for RNA**

*****QIAGEN: cat# 74004; 74404; 56304; 56504.**

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