



Isolation of single CTC from CellSearch cartridges

Version 1.0

February 2019

Scope

This protocol is for the isolation of single CTC from CellSearch cartridges using VyCAP's Puncher platform.

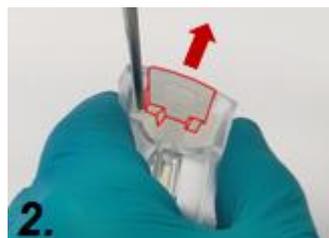
Reagents and materials

- PBS 1x, filtered
- Eppendorf tube (1.5mL)
- Long glass Pasteur pipettes or gel loading pipet tips

Isolating CTC from the sample according to the CellSearch® protocol

Process the sample according to the CellSearch® protocol. After scanning the sample with the CellTracks Analyzer II® (Menarini, Bologna, Italy), the cartridge is removed from the MAGNEST® cartridge holder and can be stored at 4°C.

Transferring MAGNEST cartridge contents



Perform the following steps subsequently.

1. Remove the cartridge out of the MAGNEST® cartridge holder immediately after scanning.
2. Remove the cartridge plug (red lining) by pushing it upward from the back of the cartridge.

Note: it is difficult to reach behind the plastic holder to be able to push the plug out of the cartridge. Use a tool, such as a pair of tweezers (shown) to push the plug out.

3. Wash the sample out of the cartridge by pipetting the sample up and down inside the cartridge using a Pasteur pipette or a gel loading pipet tip (shown). Do this at least ten times. This will homogenize the sample and detaches the cells from the cartridge.
4. Transfer the contents to an Eppendorf tube.



5. Pipet 300 μ l of 1x PBS (filtered) against the front side inside of the emptied cartridge (glass side, side of barcode). Once again, pipet this fluid up and down for ten times, rinsing the surface. The removal of the cells can be checked by placing the cartridge glass-slide up under a standard upright fluorescence microscope.
6. Add the 300 μ l PBS wash into the same Eppendorf tube as the sample was previously transferred into. Final volume of the sample inside the Eppendorf tube is 600 μ l. The 600 μ l is next transferred to the VyCAP microwells according to VyCAP's Cell Seeding protocol.
7. Sample is scanned on VyCAP's Puncher system and CTCs are isolated.