Circulating Tumor Cell Enumeration

Clonal Expansion

Circulating Tumor Cells

Single Cell Analysis

www.vycap.com
Circulating Tumor Cell Enumeration

VyCAP’s solution for the identification and enumeration of CTC is characterized by its ease of use, limited hands-on time and high recoveries. It is based on filtration and uses the larger size and rigidity of CTC compared to other blood cells to collect these cells. The difference of these two parameters between CTC and other blood cells is however small and it requires very precisely defined filtration pores to collect the CTC from the blood. Our filters are made from silicon which provides a well-defined filter that shows no auto-fluorescence and allows to acquire high quality fluorescence images of the collected CTC.

The technology consists of:

1. A filtration disposable, that contains a microsieve filter with precisely defined pores (5.0 ± 0.2µm)
2. An imaging system, to image and enumerate the collected CTC

VyCAP offers a simple solution for researchers in university hospitals and research institutes for the enumeration and analysis of CTC in whole blood of cancer patients and xenograft models.

Unique Selling Points
- Short (<1hr) and simple workflow to enumerate CTC from whole blood
- High CTC collection efficiencies >70%
- Validated protocols for CTC detection using Transfix (Cytomark) and CellSave (Silicon Biosystems)
- Perfect images due to the atomically flat microsieve filter surface without auto-fluorescence
- Dedicated imager with software for CTC enumeration
CTC filtration technology

Workflow

Sample collection
- Human: blood collection in Tansfix (Cytomark) and CellSave (Silicon Biosystems) tube
- Mice: EDTA / Heparine

Filtration
- Filter whole blood, non pre-processing required
- Flow rate 1 – 5 ml / min

Image acquisition
- Label the cells with fluorescence labels
- Acquire images, 10 – 15 minutes for the VyCAP microsieve chip

CTC analysis
- Enumeration CTC (DNA+, CK+, CD45+)
- FISH on filter
- Compatible with automatic CTC enumeration software ACCEPT

1. Collect sample
2. Filtration
3. Transfer the slide for cell labelling
4. Acquire images
5. Enumerate the CTC
VyCAP automated imaging system

Easy mounting of the filtration slide
Adaptors for
- Microscope slide
- PCR plates
- Custom sample sizes

Nikon objectives
10X, 20X, 40X

Lumencor
Sola SE II

Hamamatsu
Orca FLASH II
Scientific camera

Automatic filter cube changer
Auto-focus
Dedicated software

VyCAP automated imaging system

Fluorescence labelling

After the slide has been removed from the filtration unit it is transferred to the CellStainer. Labelling reagents, wash- or permeabilization buffers, are added on the microsieve. After incubation the excess reagents are removed by pushing the sieve onto the absorber.

Immunofluorescence labels, wash buffer, fixation buffer
Fish probes, ....

Microsieve chip
Absorber

Step 1
Incubate
Step 2
PUSH
Step 3
Enumeration

Circulating tumor cells in Human

Fluorescence images of CTC and leukocytes on a microsieve filter

Recovery SKBR-3 and PC3 cells spiked in CellSave blood from different donors

Circulating tumor cell in Mice

Graph displays the recovery of 4T1, MV3, HT1080 and B16 cell lines that are commonly used in mice tumor model systems.

150µl Heparin / EDTA blood was acquired from the mice via heart puncture or mandibular vein. The blood samples were spiked with known cell numbers. The blood was filtered followed by imaging and enumeration of the CTC collected on the microsieve filter.
CTC enumeration

Software VyCAP imaging system

VyCAP imaging system is designed for scanning the microsieve filter using maximum 6 fluorescence imaging channels. It uses standard protocols for CTC counting based on the DNA+, CK+ and CD45-, but has the ability to add additional labels against for example PDL-1 or MUC-1. The stage has 8 VyCAP slide positions. Scanning a filtration slide in 4 fluorescence colors takes 15 minutes. The software is simple and straightforward.

Besides scanning the VyCAP filtration slides, it has the ability to scan standard microscope slides, 96 well plates, C1 Fluidigm cartridge or other sample formats. VyCAP can supply adaptors for your type of sample on request.

ACCEPT CTC enumeration software

ACCEPT is designed for automatic enumeration of CTC from fluorescence images using sophisticated image analysis routines. The software was developed by our CANCER-ID partner University of Twente, chair MCBP.

- VYCAP fluorescence images are compatible with ACCEPT
- ACCEPT generates a gallery of CTC candidates to the user who makes the final selection
Products

Imaging system

The imaging system is based on a Nikon Ti-2 inverted fluorescence microscope. It is equipped with a high end scanning stages, Lumencor LED excitation light source and a Hamamatsu CMOS scientific camera to acquire high quality images with a high fluorescence sensitivity. It comes with dedicated software that enables the user to acquire images of microsieve filters, microscope slides, 96 well plates and can be customized to scan other formats as well.

<table>
<thead>
<tr>
<th>Product</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puncher system</td>
<td>Ti2-PS</td>
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</table>

Pump unit

The pump-unit is used in combination with the filtration disposable. The pump creates a negative pressure at the waste side of the disposable. The system has an adjustable pressure from 0 – 250 mbar.

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<th>Product</th>
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<tbody>
<tr>
<td>Pump unit (0 – 250 mbar)</td>
<td>PU-250</td>
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Filtration disposable

The filtration disposable contains a slide with the microsieve filter for filtration. The microsieve filter contains 150,000 pores with a diameter of 5.0 ± 0.2 µm, contained in a filter surface of 8 x 8 mm. Maximum volume 40 ml.

<table>
<thead>
<tr>
<th>Product</th>
<th>Code</th>
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<tbody>
<tr>
<td>Filtration disposable (24 units)</td>
<td>FS-510</td>
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</tbody>
</table>

Cellstainer

The Cellstainer is an indispensable need for labelling the cells that are collected onto the microsieve filter. Cellstainer absorbers are supplied with each ordered filtration disposable.

<table>
<thead>
<tr>
<th>Product</th>
<th>Code</th>
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<tbody>
<tr>
<td>Cellstainer</td>
<td>SH-60 / SH-70</td>
</tr>
</tbody>
</table>

Coverglasses

To obtain high quality images and to protect the sample from degradation, a cover glass is mounted on the microsieve filter after completion of the labelling. VyCAP cover glasses that match with the dimension of the microsieve filter. A box contains 100 cover glasses.

<table>
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<tr>
<th>Product</th>
<th>Code</th>
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<tbody>
<tr>
<td>Cover glasses (100 pieces)</td>
<td>CG-10</td>
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