Dynabeads® for Accurate Detection in Minimal Residual Cancer Research

- Tumour cell enrichment directly from whole blood or bone marrow in only 90 minutes
- Enrich viable cells for analysis and visualisation
- Create a reusable solid-phase cDNA library for tumour cell characterisation
- Highly sensitive, accurate and reproducible methods

Dynal Biotech has combined proven magnetic separation technology with tumour cell analysis to provide clear, sensitive and accurate methods to detect low numbers of tumour cells for minimal residual cancer research. The methods are rapid and simple to perform. Furthermore, as cell separations are performed in tubes and not columns, the system is gentle on cells and is efficient, ensuring high cell viability and yield.

Tumour cell detection methods are based on a two part procedure :

i) Enrich tumour cells directly from whole blood, bone marrow or MNC samples.

ii) Analyse enriched tumour cells for characterisation and confirmation.

Tumour cell enrichment is achieved using Dynabeads®, which are uniform, monodisperse, superparamagnetic polymer spheres that are coated with antibodies against cell specific markers. When Dynabeads® are added to a starting sample, the beads bind to the target cells (fig.1). These bead:cell complexes are then separated from the sample with a magnet (Dynal MPC®). Dynabeads® products offer total flexibility for enrichment. Positive or negative enrichment techniques can be used (fig.2).

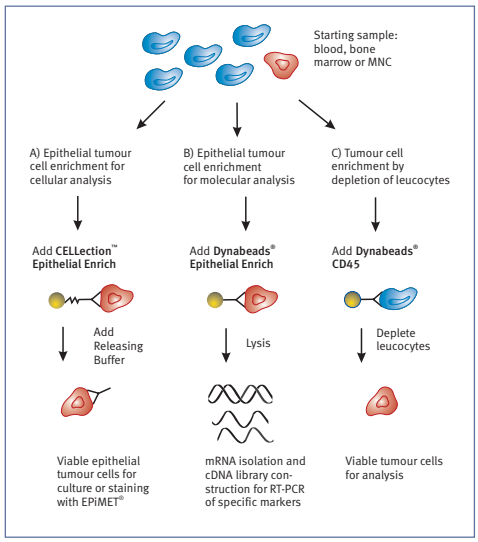

Fig. 2: Dynabeads® based enrichment options provide bead free, viable cells for cellular analysis or enriched cells for analysis of specific markers by RT-PCR or PCR (1,2).

- *Enrichment increases the sensitivity of staining alone, avoids false negative results and reduces the number of slides to be screened*
- *Enrichment eliminates the leucocytes that contribute false positive signals during nucleic acid amplification*

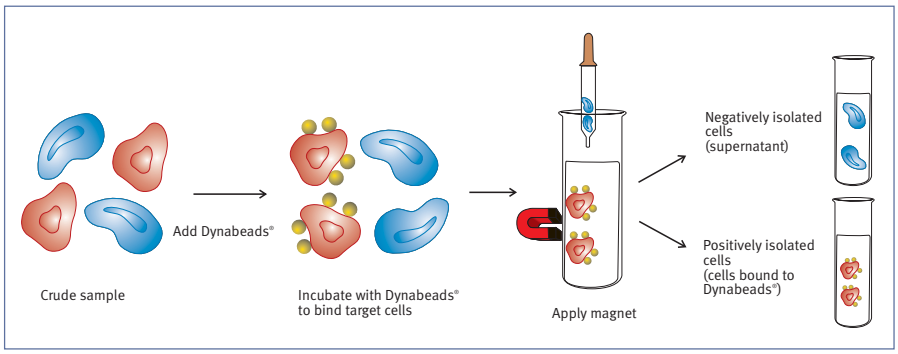

Fig. 1: Tumour cell enrichment

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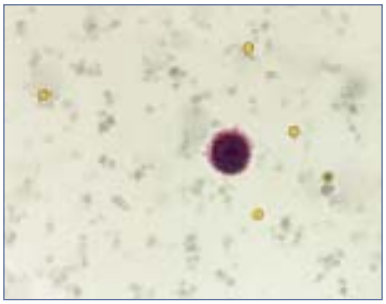

Fig. 3: Epithelial tumour cell isolated from 5ml whole blood using 250 µl CELLection™ Epithelial Enrich, detached and stained with EPiMET® (Micromet AG, Germany).

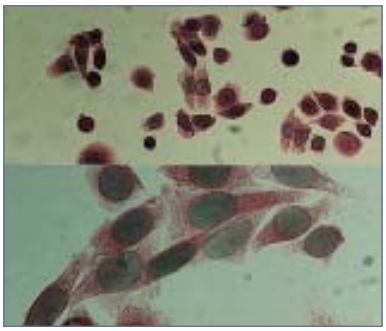

Fig. 4: Epithelial cells isolated from blood sample and cultured for eight days.

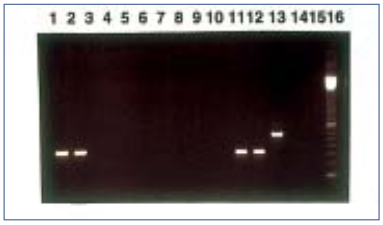

Fig. 5: Tumour cell enrichment and RT-PCR using 5 ml whole blood and 1525 µl Dynabeads® Epithelial Enrich. Enriched cells were lysed and 20 µl Dynabeads® Oligo(dT)$_{25}$ from the Dynabeads® mRNA DIRECT™ Micro Kit were used, followed by one-tube RT-PCR. Nested CK$_{19}$ RT-PCR products of seeded and unseeded whole blood, after immunomagnetic enrichment and direct mRNA isolation.

Lane 1, 2: *10 SW480 cells seeded in 5 ml of whole blood*
Lane 3-10: *5 ml whole blood, without SW480 cells*
Lane 11, 12: *Pos. control, 10 SW480 cells directly*
Lane 13: *genomic DNA, pos. PCR control*
Lane 14, 15: *neg. PCR control*
Lane 16: *MW, 100bp ladder*

A) Epithelial Tumour Cell Enrichment For Cellular Analysis

- >5 log enrichment of tumour cells
- >70% yield in spiking experiments
- Sensitivity of 1 tumour cell in 2 x 10^6 MNC
- Enrichment increases the sensitivity of ICC to equal that of RT-PCR (1)

CELLection™ Epithelial Enrich is designed to optimally enrich bead-free, viable epithelial tumour cells. CELLection™ Dynabeads® have a DNA linker between the antibody and bead surface which provides a cleavable site for cell detachment. The monoclonal antibody on these beads is BerEP4 (EpCAM Ab) against human epithelial cells (5,6,19).

Starting with a 5 ml blood sample (or 1 - 2 ml bone marrow), add 250 µl CELLection™ magnetic beads coated with BerEP4. Epithelial cells bind to the beads in a 30 minute incubation. Detach the cells from the beads with the DNase Releasing Buffer supplied. Epithelial tumour cells can then be stained using EPiMET® which detects cells expressing cytokeratins with the monoclonal antibody A45-B/B3 (fig.3). Only one single spot slide is required per sample. Enriched cells can also be grown in culture (fig.4).

Whole bone marrow samples

The CELLection™ system is a powerful tool to enrich tumour cells directly in bone marrow, giving higher sensitivity than a MNC based system. Avoiding MNC preparation reduces the risk of losing tumour cells. In spiking experiments, 100% increase in tumour cell recovery is achieved in bone marrow compared to MNC samples (3).
Successful detection of rare epithelial cells from human ascites fluid is also achieved using CELLection™ Epithelial Enrich (19).

B) Epithelial Tumour Cell Enrichment For Molecular Analysis

- Sensitivity of 1 tumour cell in 2 x 10^6 MNC or 1 tumour cell per ml of blood
- Illegitimate transcription from leucocytes is avoided
- Analyse markers such as CK19, EGP-2, PSA, k-ras, and Muc-1 (9-13,18,21)

Dynabeads® Epithelial Enrich and Dynabeads® mRNA DIRECT™ Micro Kit enrich and then isolate mRNA from epithelial tumour cells. Dynabeads® Epithelial Enrich are coated with monoclonal antibody BerEP4 (EpCAM Ab) against human epithelial cells (5,6).

Starting with a 5 ml blood sample (or 1 ml bone marrow) add 125 µl of Dynabeads® coated with BerEP4. Epithelial cells bind to the beads in a 30 minute incubation. Lyse the enriched cells with the Lysis Buffer supplied in the Dynabeads® mRNA DIRECT™ Micro Kit. Add 20 µl Dynabeads® Oligo(dT)$_{25}$ to capture poly A+ mRNA. From the captured mRNA, a solid cDNA is synthesised directly on the Dynabeads® Oligo(dT)$_{25}$ which can be used as a template for PCR amplification. The solid phase cDNA can be reused in several reactions with different primers (14,15). As the number of cells from a positive sample is less than 100 cells, nested primer sets must be used.

C) Tumour Cell Enrichment By Depletion Of Leucocytes

- Enrich any non-haematopoietic tumour cell
- Tumour cell heterogeneity does not influence enrichment
- Tumour cells with no identified specific cell surface markers can be enriched
- 2 log enrichment of tumour cells

Deplete leucocytes from an MNC sample with Dynabeads® CD45. The Dynabeads® are coated with monoclonal antibody to the leucocyte common antigen (CD45). When added to the sample the unwanted cells are captured and removed on the beads. Tumour cells remain in the supernatant and are viable and bead free.

For such a negative isolation approach, prepare MNC carefully . Resuspend MNC at 2 x 10^7 in 1 ml PBS/BSA and add 250 µl Dynabeads® CD45. After 30 minute incubation the bead:leucocyte complexes are removed with the Dynal MPC®. The viable, enriched tumour cells can be analysed. Epithelial tumour cells enriched in this way can be stained with EPiMET®.

Negative isolation has been successfully used to enrich tumour cells from patients with head and neck cancer (7), breast cancer (8,20) and bone marrow (20). Enrichment allows the examination of larger numbers of MNC with improved sensitivity, reproducibility and quantification and gives a 4 fold higher number of positive cells thanwith standard immuno-cytochemistry alone (8).

Enrichment Compared To Conventional Techniques

Enrichment and analysis	Conventional techniques
Enrichment plus ICC **Method** · Enrich tumour cells directly from 5ml whole blood or 1 - 2 ml bone marrow · Analyse enriched cells on 1 slide **Benefits** 1. Screen a larger sample volume (hence cell number) to increase sensitivity. 2. No MNC preparation so tumour cells are not lost. 3. Only 1 slide is needed as background blood cells are removed, dramatically reducing analysis time.	**ICC alone** **Method** · Prepare MNC · Analyse 10^6 MNC **Limitations** 1. Tumour cells can be lost preparing MNC by density centrifugation. Tumour cells are very heterogeneous in size and can remain in the granulocyte fraction (7). 2. Only screening 10^6 reduces the probability of being able to detect rare tumour cells. This can lead to false negative results.
Enrichment plus RT-PCR **Method** · Enrich tumour cells directly from 5ml whole blood or 1 ml bone marrow · Isolate mRNA for cDNA synthesis and PCR amplification **Benefits** 1. Screen a larger sample volume (hence cell number) to increase sensitivity. 2. No MNC preparation so tumour cells are not lost. 3. Avoid false positives as blood cells that contribute signal in PCR are removed. 4. A solid phase cDNA can be used in several PCR amplifications of multiple markers.	**RT-PCR alone** **Method** · Prepare leucocytes from blood (e.g. MNC) · Isolate RNA for RT-PCR **Limitations** 1. False positive results due to illegitimate transcription of marker genes in blood cells. 1 blood cell in 50000 will express CK[9] therefore a positive signal will always be generated if the sample contains many blood cells (13, 16). 2. Pseudogene amplification is a common problem seen with many primer sets used (17).

References

Enrichment and ICC

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Standard RT-PCR limitations

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Ordering information

Product	Samples	Product No.
CELLection™ Epithelial Enrich Contains CELLection™ Dynabeads® coated with BerEP4 and Releasing Buffer. Samples: 2 x 10^7 MNC in 1 ml 5 ml whole blood or 1-2 ml bone marrow	40	162.03
Dynabeads® Epithelial Enrich Contains Dynabeads® coated with BerEP4. Samples: 2 x 10^7 MNC in 1 ml 5 ml whole blood or 1ml bone marrow	40	161.02
Dynabeads® mRNA DIRECT™ Micro Kit For mRNA isolation for RT-PCR amplification. Contains Dynabeads® Oligo$(dT)_{25}$ and buffers	100	610.21

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