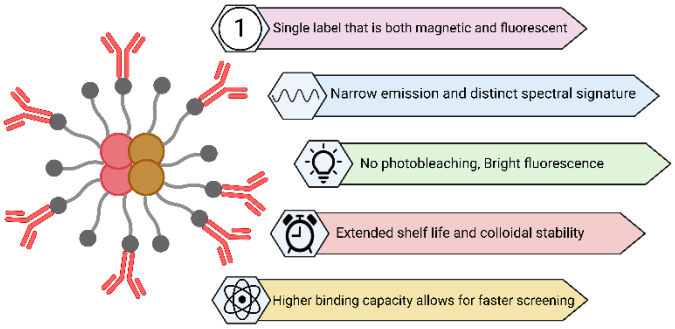
EGFR Positive Breast Cancer Cell Enrichment using MagDot Anti-EGFR

Epidermal growth factor receptor (EGFR) is a part of the receptor tyrosine kinase family which is overexpressed in various solid tumors including breast cancer and remains a stubborn therapeutic target for treatment.[i] This has necessitated the high throughput testing of drugs and other molecular compounds that can offset the high expression levels of EGFRs. Consequently, targets like these impose the need for a consistent supply chain of cells with high purity to facilitate these tests.

Access to purified cell populations has been difficult due to the absence of a straightforward, high throughput and economical cell sorting and purification procedure.[ii] While Fluorescent Activated Cell Sorting (FACS) is the gold standard for cell separation and isolation; high amounts of debris and extracellular matrix interfering with the organic dye signals through autofluorescence[iii] , low cell yield, especially in cells with rare homogenous cell receptors on target cells makes FACS challenging[iv]. Magnetic-activated cell sorting (MACS) has resolved these challenges to some extent, however, the overall separation and analysis workflow remains cumbersome, highly complex with subpar cell recovery and purity.[v]



The **MagDot** is a unique reagent that combines inorganic semiconductor nanocrystals and superparamagnetic iron oxide particles within the same nanoparticle. They have unique spectral signature, high fluorescence intensity, resistance to photobleaching and improved shelf-life stability. Owing to its dual nature, it is possible to perform cell separation on MagDot labeled cells via MACS and immediately analyze the cells through Flow cytometry or sort using FACS for further purification without any additional fluorescent labeling procedure.

CoreMag2T is an open magnetic system designed to be used with MagDot reagents. It has a field strength of 2 Tesla in the middle of the magnetic field and can hold 12 x 75 mm (5 mL) flow cytometry tubes. The inlet portion of the magnet has a rubber gasket to hold the tube firmly while being easily removable. The design enables the magnet to be kept in a half cylinder instead of a complete cylinder. As a result, the user can visualize the separation and confidently remove the supernatant.

EGFR Cell Enrichment

In a proof-of-concept experiment, MagDot conjugated to anti-EGFR was used for the magnetic separation/enrichment of EGFR-positive cells from a breast cancer tumor sample and immediately analyzed using flow cytometry. A breast cancer biopsy positive for EGFR was purchased from iSpecimen (ispecimen.com). The 68-year-old female patient who had been on hormonal therapy (but not chemotherapy) was deidentified and had consented to tissue banking. The tumor was grade 2, moderately differentiated with a Gleason score of 5,6. The specimen was collected in 2019 and live cryo-preserved.

Materials

- Breast cancer tissue from iSpecimen
- Staining Buffer: PBS + 0.5% BSA + 2 mM EDTA
- 15 mL tissue grinder (Fisher brand #02-542-09)
- 70 µm cell strainer (Falcon #352350)
- Hemocytometer
- Mouse anti-human EGFR antibody-PE Conjugate (Abcam #30738)
- MagDot 610 Anti-EGFR
- CoreMag2T
- 12x75 mm round bottom polystyrene tubes, 50 mL centrifuge tube
- Centrifuge, Vortex, Flow Cytometer

Methods

1. The tumor was thawed and transferred to a tissue grinder along with 3 mL of staining buffer.
2. The tissue was mechanically ground for 5 minutes until a clear cell slurry was visible.
3. The slurry was filtered using a 70 µm cell strainer to obtain a single-cell suspension.
4. Cells within suspension were counted using a hemocytometer, concentration was adjusted to 10^6 cells/ml, and aliquoted into three 5 mL tubes with 1 mL each.
5. Cells in each tube were then centrifuged at 1800 RPMs for 7 minutes, the supernatant was discarded, and pellet resuspended in 200 µL buffer.
6. The first tube marked as 'Unstained sample ' was used as the unstained control for flow cytometry.
7. To the 2nd tube, marked as 'PE sample', 10 µL of a mouse anti-human EGFR antibody PE- conjugate was added, and incubated for 25 minutes in dark.
8. To the 3rd tube, marked 'MagDot sample', 80 uL (100 µg iron oxide, 1.25 mg/mL) of MagDot 610 Anti-EGFR was added and incubated in ambient lighting at room temperature for 25 minutes.
9. Post incubation (in step 8), 150 µL of cell suspension from the 'MagDot sample' tube was transferred to a separate tube marked as 'MagDot Feed' (for magnetic enrichment in step 11)
10. The remaining cells in the 'MagDot Sample' from step 8 and 'PE sample' from step 7 were made up to 1 mL with buffer; washed and resuspended in 350 µL of buffer and refrigerated until flow cytometry.
11. The 'MagDot Feed' tube (from step 9) was made up to 500 uL with staining buffer and placed in a CoreMag2T magnet for 20 minutes.
12. Post 20 minute-separation, the non-magnetic fraction was aspirated and transferred into another tube marked as 'negative fraction'.
13. The 'MagDot feed' tube was removed from the magnet, resuspended in 350 µL of staining buffer and marked as 'positive fraction'.
14. Samples were immediately run on a BD-LSR II flow cytometer.

Result and Discussion

PE stain was chosen as a control for labeling EGFR-positive breast cancer cells in the tumor sample, against MagDot. Flow cytometry analysis of PE and MagDot samples revealed similar labeling percentages for both reagents (Figure 1).

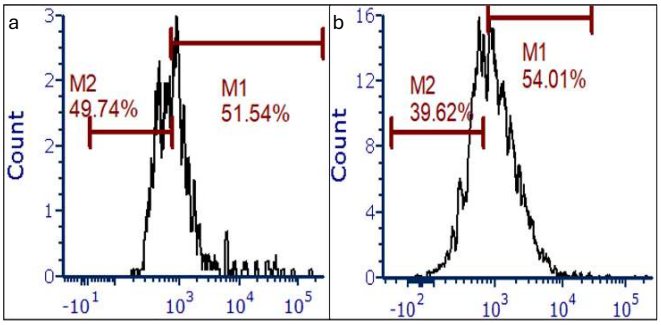


Figure 1. Flow cytometry analysis of EGFR-positive breast cancer cells stained with a) MagDot 610 and b) PE

Subsequently, the MagDot labeled cells were 98.2% positive for EGFR in the magnetic (positive) fraction (Figure 2) , with 9.84 % positive in the negative fraction. This demonstrates the enhanced enrichment capabilities of a MagDot in a single step which is in stark contrast to the purity observed with conventional reagents after multiple rounds.

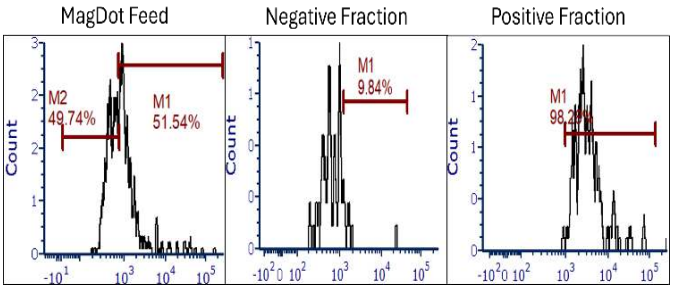


Figure 2. Enrichment of EGFR-positive cells labeled with MagDot610 Anti-EGFR in the CoreMag2T magnet. A) MagDot feed, B) Negative fraction and C) Positive fraction

Along with the improved separation, the convenience of a single label for both magnetic separation and flow cytometry simplified the workflow and enhanced the robustness of the entire procedure.

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[v] Cansu Gorgun and others, 'Synergistic Role of Three Dimensional Niche and Hypoxia on Conservation of Cancer Stem Cell Phenotype', *International Journal of Biological Macromolecules*, 90 (2016), pp. 20–26, doi:10.1016/j.ijbiomac.2015.12.053.

855-278-2266
info@corequantum.com
www.corequantum.com