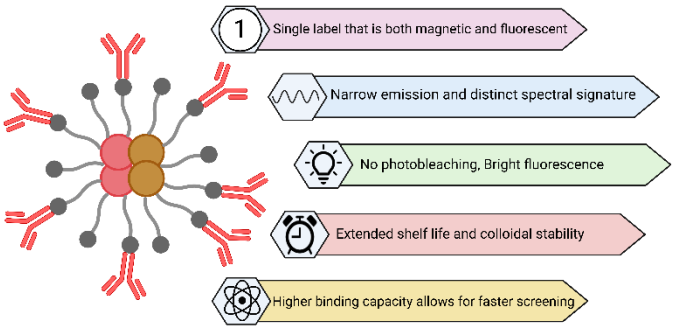
Yeast Surface Display Antibody Screening using MagDot Anti-Biotin

Yeast Surface Display (YSD)

YSD is used for the expression and subsequent screening of desired proteins and peptides on yeast cells, for antibody discovery and development.[i] Coupled with cell sorting techniques, YSD has allowed researchers to isolate antigen-specific antibodies (or antibody fragments) from a library of clones, with high affinity and specificity.[ii]

The current isolation technique is tedious since cell separation involves multiple rounds of enrichment and labeling steps to acquire the desired protein with sufficient purity. Here, we detail a protocol that uses MagDot Anti-Biotin for rapid and effective separation and enrichment of antigen-binding clones in a 2 Tesla open magnetic system.



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 Magnet Activated Cell Sorting (MACS) and immediately analyze the cells through Flow Cytometer or sort using Fluorescent Activated Cell Sorting (FACS) for further purification without any additional fluorescent labeling procedure.

CoreMag2T is an open magnetic system designed to be used with MagDots. It has a field strength of 2 Tesla in the core 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. The user can visualize the separation and confidently remove the supernatant.

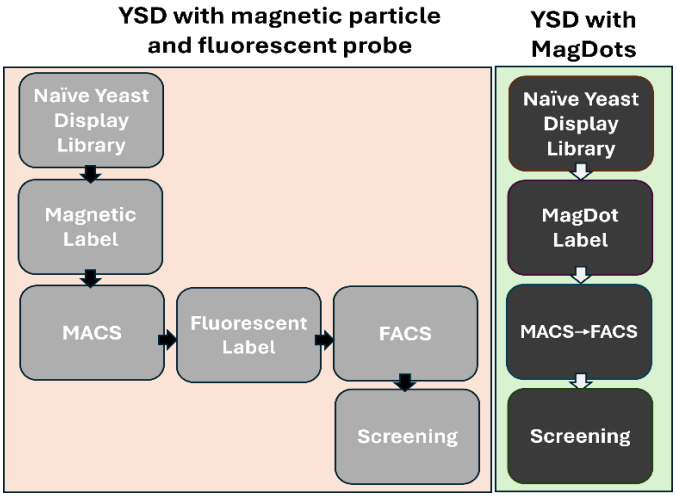


Figure 1. Standard multi-step protein screening from YSD vs single step screening with MagDot

Yeast Display Nanobody Library Screening was performed to screen for engineered yeast strain using Anti-Biotin conjugated MagDot with an emission maximum of 610 nanometers (MagDot610). MagDot was used to label a biotinylated ALFA-tagged fluorescent protein (mEos3.1)[iii] bound to yeast cells displaying anti-ALFA nanobody and separated from yeast cells devoid of anti-ALFA nanobody (Figure 2).

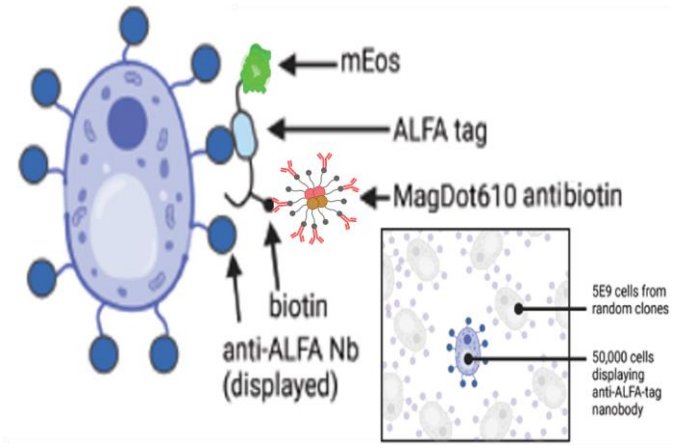


Figure 2 MagDot with Anti-Biotin bind to biotinylated ALFA-tag , Yeast strain expressing anti-ALFA nanobody (positive control) mixed with yeast cells from random clones (inset).

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Materials

- Yeast Display Nanobody Library
- MagDot 610 Anti-Biotin
- Biotinylated Antigen
- Anti-HA Alexa Fluor 647
- CoreMag2T
- Selection Buffer (20 mM HEPES, pH 7.5; 150 mM sodium chloride, 0.1% (w/v) BSA, 5 mM maltose)
- Propidium Iodide (PI)

Methods

1. Suspend yeast cells in 5 mL selection buffer (1E+09/mL) with appropriate diversity of nanobody expression.
   Note: More than one wash can be performed to ensure traces of previous media have been removed completely.
2. To remove non-binders from the yeast cell suspension, add 250 µL of MagDot 610 Anti-Biotin to the cells and incubate for 30 minutes at 4ºC.
3. Centrifuge cells for 5 minutes at 4000 rpm at 4ºC, discard supernatant.
4. Resuspend the cells in a 5 mL selection buffer and place it in CoreMag2T for 15 minutes.
5. With the tube still in the magnet, transfer the supernatant to a fresh tube.
6. Centrifuge cells in supernatant for 5 minutes at 4000 rpm, 4ºC and discard supernatant.
7. Resuspend the cells in a 5 mL selection buffer.
8. Add biotinylated antigen (*with ALFA-tagged fluorescent protein (mEos3.1)*) to the cell suspension and incubate with mixing for 1 hour at 4ºC.
9. Centrifuge cells for 5 minutes at 4000 rpm, 4ºC and discard supernatant to remove unbound antigens.
10. Resuspend cells in 5 mL selection buffer, centrifuge again for 5 minutes at 4000 rpm, 4ºC and discard supernatant.
11. Resuspend in 5 mL selection buffer, add 250 µL **MagDot 610 Anti-Biotin** and incubate for 30 minutes at 4ºC. This is for the selection of MagDot labeled yeast cells.
    Note: If additional epitopes are to be visualized in a cytometer, add fluorescent antibodies simultaneously with MagDot. For the library utilized in this protocol, anti-HA-Alexa Fluor 647 antibodies were added to stain the displayed nanobodies.
12. Centrifuge cells for 5 minutes at 4000 x rpm, 4ºC and discard supernatant.
13. Resuspend in 5 mL selection buffer and place the tube in CoreMag2T for 15 minutes.
14. Remove supernatant, resuspend immobilized cells with 5 mL selection buffer and repeat the magnet separation in CoreMag2T for another 15 minutes.
15. Remove supernatant and resuspend immobilized cells in 400 µL selection buffer containing 1 µg/mL PI for analysis.
16. Sort cells with FACS and recover into media.
17. Perform Next Generation Sequencing (NGS) to screen for the clones of interest.

Result and Discussion

MagDot610 Anti-Biotin was first assessed by magnetically isolating anti-ALFA nanobody displaying yeast cells present at a concentration of 1% in random clones displaying yeast, with CoreMag2T magnet. BD FACSymphony S6 Cell sorter was used to separate MagDot labeled cells. Enrichment of 70 % was achieved in a single magnetic separation step with the MagDot, while eliminating the need for a separate labeling step and enabling direct flow cytometry analysis after sorting (Figure 3).

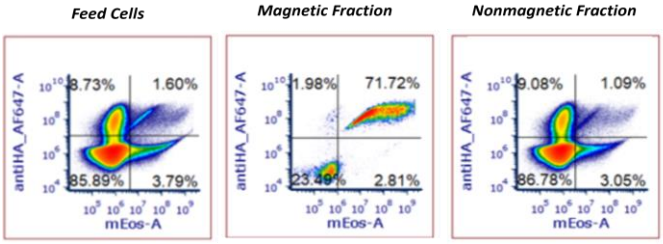

Figure 3. Flow cytometry analysis of 1% doped ALFA-Nb displaying cells. Feed cells, Magnetic Fraction and Non-Magnetic Fraction are shown.

To further test for the highest degree of cell purity that can be attained with the MagDot, the engineered yeast strain was doped as a positive control (50,000 cells) into a background of negatives (five billion cells) at 0.001%. The positive control cells were enriched using MagDot610 Anti-Biotin in CoreMag2T magnet. The enriched cells were then sorted immediately using FACS for live cells and recovered for Next Generation Sequencing (NGS). NGS revealed doped strain as the top hit and was read three times more than the next

abundant clone (Figure 4). This highlights the superior separation efficiency and resulting purity of cells that can be achieved by using MagDot.

In this proof-of-concept antibody screening using engineered yeast strain, MagDot eliminated the use of multiple reagents, simplified the workflow by eliminating the need for multiple rounds of selection and consequently reduced the screening time from 5 weeks to a couple of days.

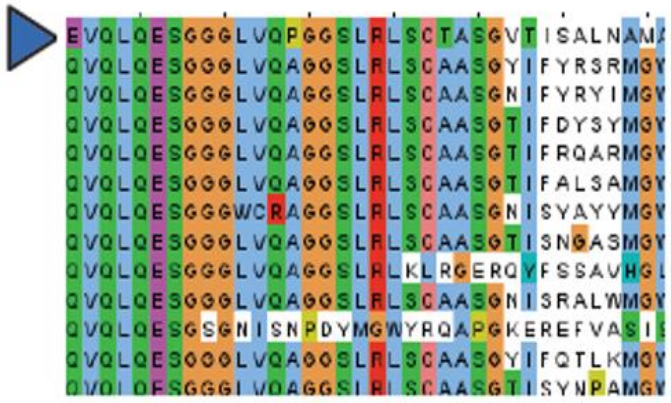

Figure 4. NGS analysis confirming the clone of interest

The high antigen-binding capacity of nanobodies combined with the enhanced cell isolating abilities of MagDots allows for an excellent high throughput platform for antibody selection with high specificity.

Acknowledgement

This study was performed by Dr. William Bret Redwine, Head- Custom Protein Resources, Stowers Institute for Medical Research

[i] Boder and Wittrup, "Yeast Surface Display for Screening Combinatorial Polypeptide Libraries."

[ii] McMahon et al., "Yeast Surface Display Platform for Rapid Discovery of Conformationally Selective Nanobodies."

[iii] Götzke et al., "The ALFA-Tag Is a Highly Versatile Tool for Nanobody-Based Bioscience Applications."