absolute antibody

Recombinant and hybridoma-derived antibodies: A case study using anti-amyloid β antibody clone WO2

Sequenced and recombinantly manufactured vs. secreted by original hybridoma

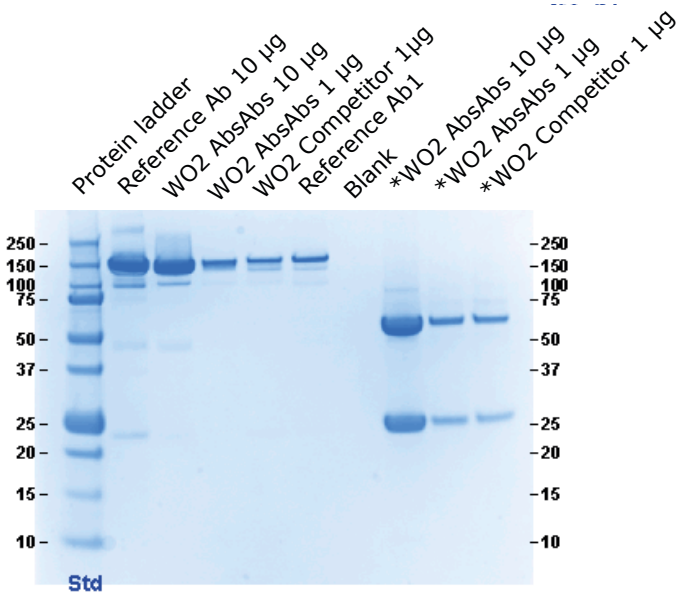
Recombinant antibodies in research have received much attention in recent years, in part as they may hold the solution to ensuring antibody reproducibility (Bradbury and Plückthun, 2015) but also because they have various other advantages such as flexibility through protein engineering as well as animal-free production (Groff *et al.* 2015).

However, many researchers worry how a recombinant antibody, with the same variable domain sequences, will perform in comparison to the hybridoma-derived parental antibody (see comments in Baker, 2015).

In particular, specificity and affinity for an antigen are two key properties of antibodies researchers readily consider. To move away from vague generalisations, we decided to compare these two properties for a recombinant antibody and its parental hybridoma-derived antibody.

We therefore generated a recombinant version of the anti-amyloid β antibody WO2 in the same format as the parental antibody (Mouse IgG2a).

To ensure a level playing field we sought to investigate how the composition of our recombinant antibody WO2 compared to that of a competitor's hybridoma-derived W02 (Competitor M).



*Reduced

Figure 1.

A Coomassie-Blue stained SDS-PAGE gel (Figure 1), with both recombinant and hybridoma derived antibodies, was run under both native and reducing conditions.

Both antibodies appeared, at a calculated 1 µg protein per lane, devoid of major contaminants. The single band in the native lanes splits up cleanly into heavy- and light-chain species under reducing conditions.

Importantly, judging by the intensity of bands, the amount of protein in the recombinant and hybridoma-derived antibody preparation was very similar.

Therefore, it should not be unfair to compare the activity of the antibody in these preparations.

To do this an immunoassay was set up as shown in Figure 2. Different amyloid β peptides were immobilized on a microplate and probed first with WO2 and then a horse-radish peroxidase labelled secondary antibody.

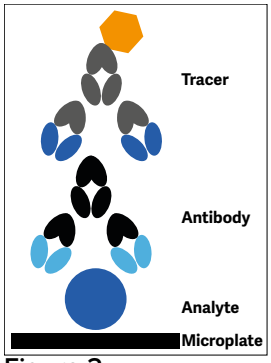


Figure 2.

First the antigen-specificity of the antibodies was compared.

The pattern of antigen recognition – the relative differences of reactivity to different antigens – was comparable between the two primary antibody preparations, with

a slightly stronger overall signal observed for the recombinant WO2 (Figure 3).

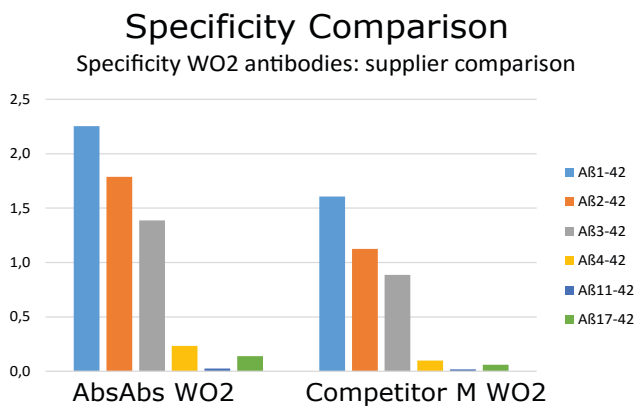


Figure 3.

These data show that recombinant production does not change the antigen specificity compared to hybridoma-derived antibody.

To see if we would consistently observe a stronger signal from the recombinant antibody, both antibodies were titrated onto Aβ 2-42 (Figure 4A) and Aβ 3-42 (Figure 4b) coated microplates.

Whilst both antibodies behaved similarly, once more the recombinant antibody consistently gave a stronger signal than the hybridoma derived antibody.

This indicates a comparable, if not higher affinity for the antigen.

This case-study of the WO2 antibody clone shows that there are no immediate drawbacks to using the recombinant over the hybridoma-derived format.

There are however advantages that come with recombinant production, such as the ability to generate different formats (e.g. Rabbit IgG version or recombinant F(ab')2 or Fab fragments).

Moreover, and perhaps addressing concerns over costs voiced in articles discussing the advantages and drawbacks of recombinant antibodies (see comments in Baker, 2015), our recombinant version of the anti-amyloid βWO2 clone retails at around 1/5th of the price of the hybridoma-derived antibody supplied by Competitor M.

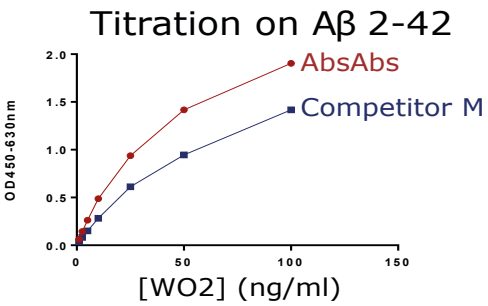


Figure 4A.

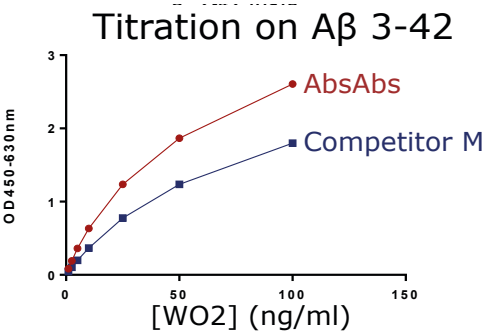


Figure 4b.

Summary:

A recombinant anti-amyloid antibody was compared to its hybridoma-derived parental antibody:

- **Similar antigen-specificity but consistently higher reactivity of recombinant antibody compared to hybridoma-derived antibody**
- **Unrivalled flexibility, providing a wide variety of different formats.**
- **Competitive pricing.**

To find out more about our ever-growing antibody catalogue, which includes 19 research-grade biosimilars, as well as recombinant versions of 'classic' antibodies – such as anti-F4/80 [Cl:3A-1] and anti-c-myc [9E10] – visit **absoluteantibody.com**, or email **sales@absoluteantibody.com**.

We also offer competitive hybridoma sequencing, antibody engineering and recombinant expression services.