Developing Anti-thyroglobulin Antibody Pairs: A Framework For Screening Against Complex Antigens

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ABSTRACT

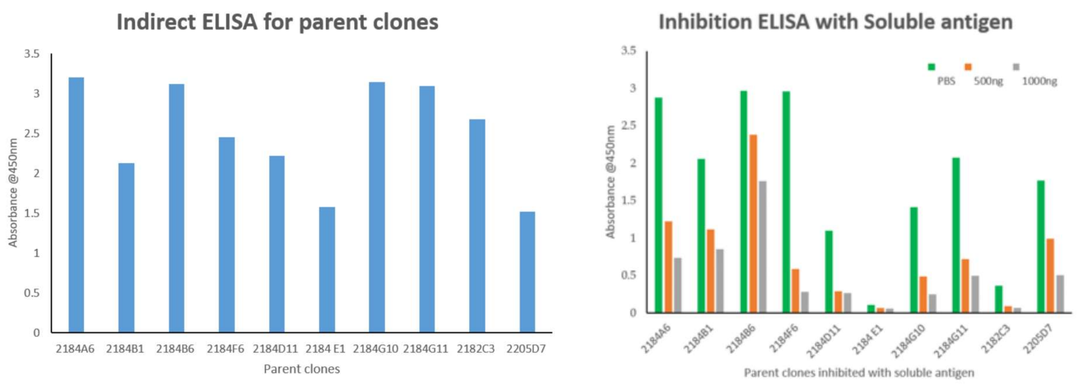
A major challenge in the development of immunoassays for any target antigen is the identification and screening of suitable monoclonal antibody (mAb) matched pair. We developed matched pair for thyroglobulin (TG), a 660 kDa homodimeric glycoprotein secreted by thyroid follicular cells, widely used as a specific biomarker in thyroid cancer diagnostics. TG's large size, multiple disulfide bonds, and high glycosylation contribute to its structural complexity and challenge assay development. In our study, several anti-TG mAbs exhibited strong reactivity in indirect ELISA but failed to function effectively as matched pair in sandwich ELISA, suggesting challenges in antibody-soluble antigen complex formation or simultaneous epitope recognition. To investigate, we performed inhibition ELISA with varying concentrations of soluble TG antigen, allowing the identification of mAbs capable of binding soluble TG. In another set of inhibition ELISA, we inhibited the binding of potential detector mAb with their matched pair. This confirmed recognition of distinct epitopes by detector and capture mAbs. Based on these findings, we hypothesized that the conformational changes in the capture antibody upon adsorption to ELISA plate might have impaired antigen recognition. To resolve this, we biotinylated the capture antibody and utilized a streptavidin-coated ELISA plate. This enabled successful identification of functional matched pair mostly by improving antibody orientation. Our findings emphasize the importance of antibody conformation in immunoassay development. Integrating inhibition ELISA and biotin-streptavidin capture strategies enables better matched pair screening, preventing the loss of potentially valuable mAbs due to conformational artifacts when developing sensitive assays for complex antigens.

METHODS

1. Immunization
2. Fusion
3. Screening for Parent Clones
4. Single Cell Cloning
5. Selected Clones
6. Adaptation in Serum Free Media
7. Antigen and Antibody Dilution
8. Purification
9. Match Pair Analysis (Sandwich ELISA & Inhibition ELISA)
10. Sandwich ELISA with Biotinylated Capture

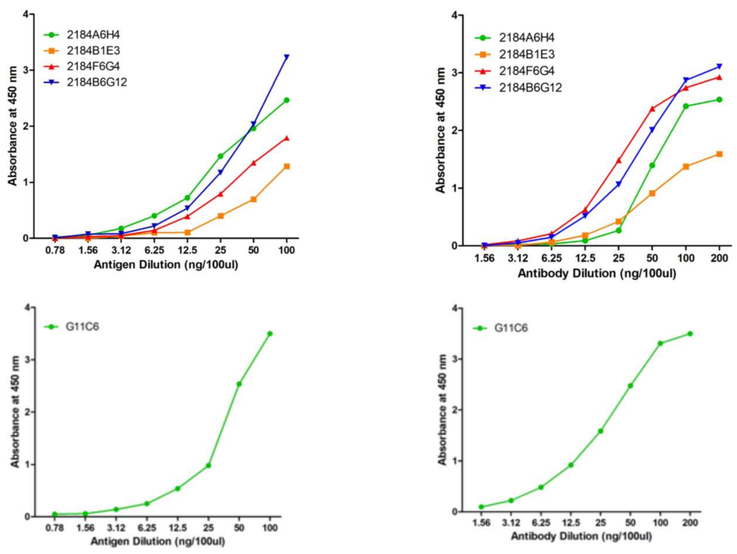
RESULTS

Screening of parent clones



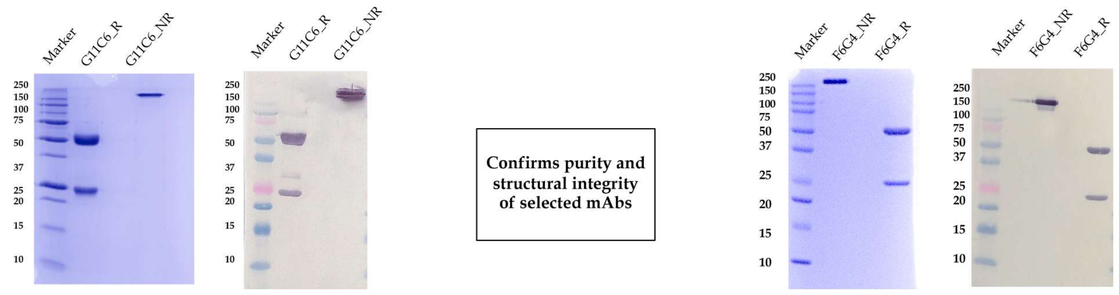
Indirect ELISA was used for identifying reactive clones and soluble-antigen inhibition verified antigen-specific binding.

Antigen and Antibody dilution curve



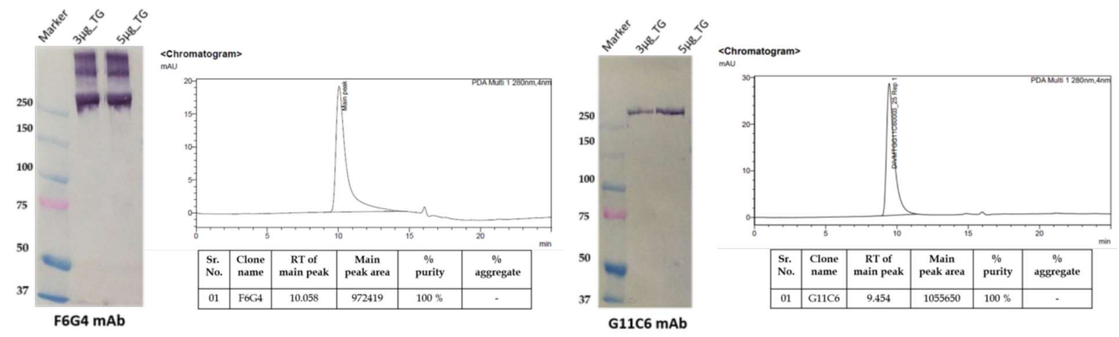
Selected mAbs show dose dependent reactivity against TG antigen with good sensitivity

SDS PAGE and Western Blot analysis



Confirms purity and structural integrity of selected mAbs

Specificity and Purity of Anti-TG mAb

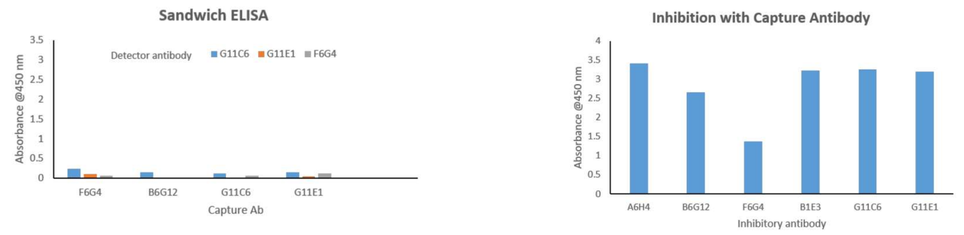


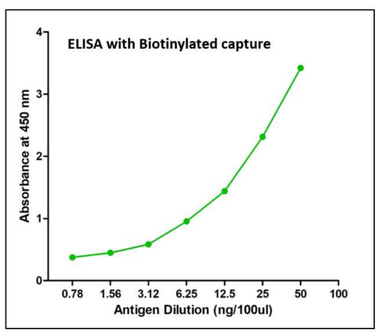
Sr. No.	Clone name	RT of main peak	Main peak area	% purity	% aggregate
01	F6G4	10.058	972419	100 %	-

Sr. No.	Clone name	RT of main peak	Main peak area	% purity	% aggregate
01	G11C6	9.454	1055650	100 %	-

Western blot confirmed the specificity of mAbs and <3% aggregation by HPLC indicates that all antibody molecules are fully functional.

Matched pair analysis





- Sandwich ELISA with selected mAbs did not yield detectable signal may be due to the overlapping epitopes between capture and detector antibody
- Inhibition ELISA with capture antibody confirmed that they have different epitopes from the detector antibody
- To check if the conformation of capture antibody affected the detection of antigen in sandwich ELISA we biotinylated the capture and used streptavidin coated plates
- We successfully obtained the matched pair for TG on using the biotin-streptavidin capture strategy

CONCLUSIONS

1. Selected anti- TG mAbs show dose dependent reactivity against TG antigen with good sensitivity.

2. SDS-PAGE, Western blot, and HPLC confirmed the integrity, purity, and specificity of anti-TG mAbs, validating their suitability for immunoassay development.

3. Inhibition ELISA demonstrated non-competing binding between certain capture–detector pairs, confirming recognition of distinct epitopes essential for matched pair formation.

4. Implementation of a streptavidin assay using biotinylated capture antibodies facilitated matched pair discovery and confirmed the critical role of antibody orientation in assay functionality.

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