Faster Generation of Anti-Drug Antibodies Using SpyTag Technology

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Introduction

Anti-idiotypic antibodies are critical reagents in the development of therapeutic antibodies, being used for the development of pharmacokinetic (PK) and immunogenicity assays. Bio-Rad generates highly specific recombinant antibodies using the fully synthetic Human Combinatorial Antibody Libraries, HuCAL PLATINUM®. The output format is a monovalent Fab antibody. For various assays, a bivalent or full-length IgG format is essential. Conversion of Fab to IgG is a time-consuming process that takes several weeks. We recently combined recombinant antibody expression with SpyTag protein ligation technology, whereby a covalent isopeptide bond spontaneously and quantitatively forms between a 13 amino acid peptide tag (SpyTag) and a 12 kDa protein (SpyCatcher).

By incorporating a SpyTag into our recombinant antibodies, we can convert a monovalent Fab fragment to a bivalent Fab or an IgG-like format in just one hour. Fab antibody fragments with a SpyTag at the C-terminus of the heavy chain were expressed in a proprietary *E. coli* strain. Antibody Fc domains with SpyCatcher (FcCatcher) were produced and used for the assembly of Ig-like molecules. Performance in various ligand binding assays was shown to be comparable to their IgG counterparts. Moreover, SpyTag technology offers fast and robust site-directed labeling of antibodies by using preproduced and site-specifically conjugated SpyCatcher modules. We have shown that the resulting antibodies perform better than conventionally labeled alternatives, resulting in more sensitive assays. Antibody candidates from selection campaigns can be easily screened in parallel as IgG-like molecules or as directly labeled antibodies without the need for labor- and time-intensive IgG conversion or conjugation reactions.

Custom Antibody Generation

- Generation of custom monoclonal antibodies for research and in vitro diagnostic use
- HuCAL PLATINUM phage display technology
- Over 17 years of experience in this business
- Generation of almost 50,000 different antibodies
- Well-established automated procedures
- Fab antibodies ready in as little as 8 weeks

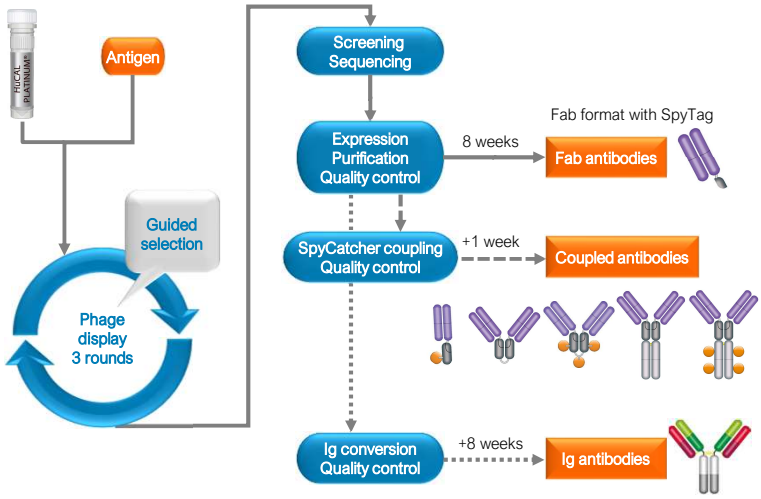


Fig. 1. Custom antibody generation service. Generation of Fab antibodies takes 8 weeks; conversion of Fab to fully human IgG takes a further 8 weeks in a time-consuming and labor-intensive process. Using our new service, coupling of a Fab with a SpyTag to a SpyCatcher generates multiple formats in only 1 additional week.

SpyTag Technology – Fast, Spontaneous Reaction

SpyTag technology is based on the SpyTag peptide and SpyCatcher protein, which are derived from the fibronectin binding protein (FbaB) of ***S**treptococcus **py**ogenes* (Spy). The FbaB protein contains an intrachain isopeptide bond between the sidechains of a lysine and an aspartic acid within the Ig-like collagen adhesin domain. When the SpyTag and SpyCatcher are mixed, the covalent isopeptide bond is formed between these amino acids. This reaction is fast, quantitative, and occurs spontaneously with high yield in diverse conditions of pH, temperature, and buffer.

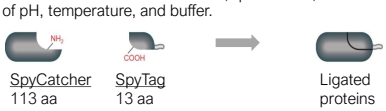
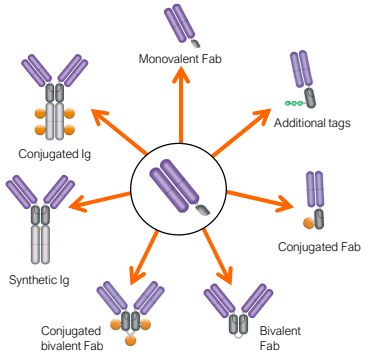


Fig. 2. (left) SpyTag-SpyCatcher protein ligation.

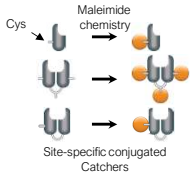
Fig. 3. (right) A monovalent Fab with SpyTag (center) can be converted to multiple different formats via the formation of a covalent isopeptide bond with the chosen Catcher, thus offering the assay developer a variety of options depending on the desired assay setup and platform to be used.



Site-Specific Conjugation of SpyCatcher Reagents

SpyCatcher2 and BiCatcher2 were modified by adding a cysteine residue, which can be used for site-specific conjugation and facilitates a controlled degree of labeling. Coupling a SpyTagged Fab to a preconjugated Catcher avoids the problem of the label interfering with the antibody-antigen binding site and leads to improved performance in the assay.

Fig. 4. Cysteine residues introduced into SpyCatcher2 and BiCatcher2 enable site-specific conjugation with a label of choice.



SpyTag Fab and SpyCatcher Coupling Reaction

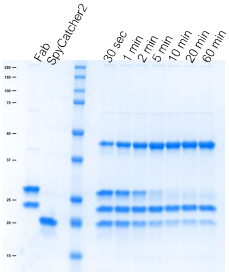
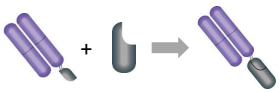


Fig. 5. Example of coupling kinetics for SpyTag-SpyCatcher reaction. His-SpyCatcher2 (#TZC001) coupling kinetic was analyzed on an AnyKD Criterion TGX Stain-Free Gel (#5671125). A SpyTagged Fab (AbD35759, Fab-F-Spy2-H format) was loaded in lane 1 and His-SpyCatcher2 in lane 2. Precision Plus Protein Unstained Protein Standard (#1610363) was run in lane 3. In lanes 6–12 the coupling reaction of the Fab with a 25% molar excess of His-SpyCatcher2 at RT was loaded. The reaction was stopped at the indicated time by addition of 4x Laemmli Sample Buffer (#1610747). All lanes were loaded with 3 µg protein.



Comparison of Antibody Format Performance in PK Bridging ELISA

Three anti-daratumumab antibody clones in the format Fab-SpyTag coupled to human IgG1-FcSpyCatcher3 (FcCatcher) or full-length human IgG1 format were compared as the detection reagent in a PK bridging ELISA (Figure 6). The assay using antibodies in SpyTag format coupled to prelabeled FcCatcher (left) shows higher sensitivity and better performance for all three clones.

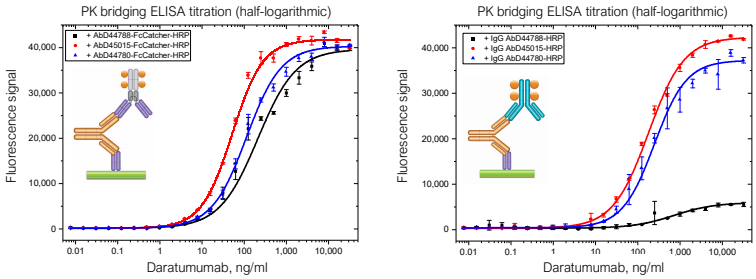


Fig. 6. Antibody format comparison in PK bridging ELISA. A microtiter plate was coated overnight with anti-daratumumab antibody (clone AbD45015, monovalent Fab format) at a concentration of 1 µg/ml. After washing and blocking with PBST + 5% BSA, PBST with 10% human serum was added spiked with increasing concentrations of daratumumab. Detection was performed using the three anti-daratumumab clones indicated, in format Fab-F-Spy2-H coupled to prelabeled hIgG1-FcSpyCatcher3-HRP (left) or in full-length HRP conjugated hIgG1 format (right), at a concentration of 0.5 µg/ml (FcCatcher-HRP) or 2 µg/ml (IgG-HRP) in HISPEC Assay Diluent (#BUF049A) followed by QuantaBlu Fluorogenic Peroxidase Substrate.

Comparison of Antibody Format Performance in ADA Bridging ELISA

A set of antibodies in monovalent Fab-SpyTag format can be rapidly converted to Ig-like format using FcCatcher for comparison as a reference standard in an anti-drug antibody (ADA) assay. In this example, three anti-daratumumab antibody clones in the format Fab-SpyTag coupled to hIgG1-FcCatcher, or in full-length hIgG1 format were compared. The performance of the three clones in the different formats is similar. This rapid modelling can help the user select the most appropriate candidates for conversion to fully human IgG for the clinical assays.

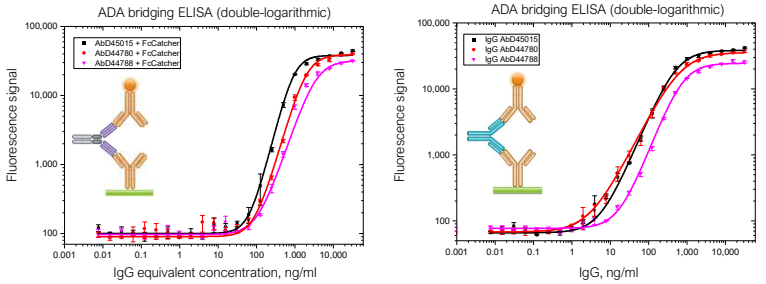


Fig. 7. Antibody format comparison in ADA bridging ELISA. A microtiter plate was coated overnight with daratumumab at a concentration of 1 µg/ml. After washing and blocking with PBST + 5% BSA, PBST with 10% human serum was added spiked with increasing concentrations of the three anti-daratumumab clones indicated, in format Fab-F-Spy2-H coupled to hIgG1-FcSpyCatcher3 (left) or in full-length hIgG1 format (right). Detection was performed using HRP conjugated daratumumab followed by QuantaBlu Fluorogenic Peroxidase Substrate.

Summary

The success of bioanalytical assays is significantly impacted by the quality of the antibodies used. Combining recombinant antibody generation using HuCAL® technology with SpyTag and SpyCatchers results in an antibody engineering toolbox that brings unprecedented flexibility to assay design. Having high quality, well-characterized, recombinant antibodies early in the development lifecycle enables the design of a selective and sensitive assay, a guaranteed supply for the duration of the study, and the generation of trustworthy data to support the regulatory approval of the biologic.

- Fast and robust site-specific labeling of custom antibodies
- Access to bivalent Fabs or Ig-like antibodies within one hour
- Fast and easy switching of isotype, including human, mouse and rabbit
- Similar or better performance of corresponding clones in various assays
- Defined conjugated product
  - Controlled degree of labeling
  - High batch-to-batch consistency
  - No modification of antibody binding site
- One recombinant Fab gives access to a multitude of experimental setups

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