SPOTLIGHT feature

Biotechnology & Immunology

Enhancing immunoassay design through antibody conjugation kits

Sharon Sanderson, PhD, Bio-Rad Laboratories, Inc

Antibodies serve as foundational tools across a broad range of research fields from immunology to oncology and infectious disease. Advances in reagent development have facilitated the generation of extensive, high-quality toolboxes of specific antibodies and compatible labels. Labels, such as fluorescent dyes, proteins or enzymes, enable target detection when conjugated to antigen-binding antibodies. Using distinct labels supports the simultaneous detection of multiple targets in a single immunoassay experiment, such as western blotting, flow cytometry, ELISA, and microscopy.

Target detection can be achieved via either a direct approach using a labelled primary antibody which recognises the target or an indirect approach which requires the use of a labelled secondary antibody specific to the primary antibody. The use of direct detection is widely preferred over indirect detection largely due to the requirement for fewer reagents, which reduces costs, simplifies assay format and design, reduces experimental time, and yields superior data and experiment reliability. It also enables the multiplexed detection of antigens from the same species.

Although many labelled antibodies are commercially available, especially fluorescent formats, coverage across clones and labels is incomplete. Less popular clones often lack suitable labels, meaning many assays still default to using an indirect format and forgo the benefits of direct detection. Consequently, researchers often design experiments based on the commercial availability of labelled antibodies rather than what's optimal. In this scenario, access to tools that allow researchers to easily conjugate labels to antibodies themselves are greatly advantageous for optimal experiment design.

Conjugation kits address labelled antibody availability limitations

Conjugating antibodies without the use of conjugation kits can take up researchers' valuable time in the lab and lead to loss of antibody. Antibody conjugation kits offer a flexible and convenient solution for the direct conjugation of labels to antibodies – with preoptimised reagents and protocols, many kits are easy to use and require minimal hands-on time, often with short protocol durations and incubation periods, or overnight incubation. The use of conjugation kits also doesn't require the use of specialised equipment, making them very accessible. Many kits are also designed to work with a broad range of purified antibodies, including different isotypes, concentrations and buffers.

By enabling the fast, reliable and reproducible labelling of antibodies, conjugation kits enable researchers to easily expand their toolkit for various research applications. This not only allows researchers to custom label their in-house antibodies but also enables the assembly of optimal panels by pairing the best antibody-label combinations for each assay rather than being limited to whatever pre-labelled clones are commercially available.

While antibody conjugation kits are useful in the context of many research applications, there are many specific cases where they might be essential. For instance, in pharma or academic settings, proprietary antibodies often cannot leave the company or institution. Conjugation kits provide a streamlined method for on-site, on-demand, custom-labelling of these antibodies with a wide range of different labels like fluorescent dyes, enzymes, and biotin/streptavidin.

Beyond these compliance-driven scenarios, conjugation kits also fill critical gaps where pre-labelled reagents are scarce – most notably in veterinary immunology. Limited availability of antibodies against veterinary antigens and few conjugated options make experimental design more challenging and constrains multiplexing. Enabling researchers to label available antibodies with suitable tags, conjugation kits expand assay choices and accelerate progress in veterinary immunology.

Real-world applications in veterinary and translational research

The utility of conjugation kits was demonstrated in a recent study investigating avian influenza virus (AIV) prevalence in wild ducks, which are considered key natural reservoirs for the virus and therefore play an important role in AIV outbreaks [1]. Immunological studies in ducks have been hindered by the lack of species-specific antibody reagent availability. In this study, conjugation kits supported the development of an automated flow cytometry-based protocol enabling the monitoring of duck immune status by detecting changes in white blood cell (WBC) counts. Specifically, selected monoclonal antibodies that recognised different types of duck WBCs (e.g., $CD4^+$ and $CD8^+$ T cells, lymphocytes, etc.) were conjugated to unique labels (e.g., FITC, PE, PerCP-Cy5.5, etc.) using a commercially available kit enabling immune system assessment via flow cytometry-based WBC quantification. This study demonstrated that age and sex have an effect on WBC counts in ducks – with male ducks naturally infected with low pathogenic AIV showing a reduction of lymphocytes and thrombocytes, which is also commonly observed in humans infected with influenza A. The researchers also showed that this protocol can be scaled to analyse samples collected from wild ducks in field studies, allowing broad immune status monitoring of typically understudied avian populations.

Similarly, conjugation kits can also be beneficial for translational research models, where human disease biology must be mirrored in relevant models. Oral melanoma is a clear example in which human and canine tumours are genetically and biologically similar, making dogs a valuable comparative model. In this model, primary cell cultures present a useful tool for characterising neoplastic cells and investigating cellular pathways that support tumour progression. One study investigated the use of fine-needle aspiration (FNA) as a less invasive technique than surgery to sample cells from canine oral melanocytic tumours and nodal metastases for primary cell cultures [2]. In the study, samples were labelled with antibodies conjugated to RPE-Cy7, enabling characterisation of melanocytic cellular phenotypes via flow cytometry. This study is the first to demonstrate the establishment of primary tumour cell cultures from FNA samples in dogs for functional studies of oral melanomas, simplifying sample collection for the development of translational in vitro models.

Conjugation kits broaden panel design and multiplex research capabilities

Beyond veterinary and translational research, antibody labelling also enhances the capabilities of flow cytometry for other research applications. Conjugation kits can expand the variety of labels conjugated to antigen-specific antibodies, resulting in bigger flow panels to support multiplexing applications. Conjugation kits also make it easier to combine multiple labelled primary antibodies, which reduces the risk of cross-reactivity that can occur when using secondary antibodies from the same host species in indirect assay formats.

The use of conjugation kits to support multiplexing was demonstrated in a recent study which investigated the role of two transcription factors, Foxn1 and Hif-1α, in the regulation of dermal white adipose tissue (dWAT) during wound healing [3]. Flow cytometry was performed on wounded skin of mice to validate the efficiency of lentiviral transgene incorporation of Foxn1 and Hif-1α into dermal adipocytes, and to characterise the cell. To enable multiplex labelling, the cells were incubated with multiple antibodies conjugated to varying labels through the use of conjugation kits. This study showed that both transcription factors cooperatively regulate skin dWAT during the proliferative phase of wound healing via the Igf2 signalling pathway. Lentiviral gene delivery of Foxn1 and Hif-1α also facilitated an increased expression of genes involved in lipogenesis and a reduction in macrophage content at the wound site.

By expanding the options of dyes that can be used in flow cytometry, conjugation kits make it easier to use a combination of bright fluorophores that give low spillover and spreading, producing high-quality data to enable clear identification of populations.

Choosing the right kit

Numerous antibody conjugation kits are available to researchers, but the best choice depends on individual applications and experimental set-up. There are a number of factors and questions researchers need to consider to help identify which conjugation kit to use (*Figure 1*). For example, not all purified antibody storage-buffer components are compatible with every type of conjugation chemistry. Specifically, the storage buffer

may contain other proteins and stabilisers (e.g., BSA) which may block reactive groups or reduce labelling efficiency necessitating buffer exchange or antibody purification prior to conjugation. However, this step is not necessary with kits that are compatible with BSA or other stabilisers in the storage buffer.

It is also important to consider how long you'll use the antibody conjugate for as the stability of the final product varies from kit to kit. If you're planning to use the antibody straight away it might not be necessary to use a kit that produces a product that is stable for more than 24 hours.

Conjugation kits are also often designed to react with a predetermined quantity of antibody, which can help researchers to plan experiments if they know how much antibody will be needed. The availability of a certain kit can also help researchers to plan experiments, for example, if they are planning long-term studies, researchers will not only need a highly reproducible conjugated antibody which can be achieved through conjugation kits, but it will also be critical that the kit is available to be repeatedly purchased. Finally, researchers looking into using conjugation kits for their research, should aim to obtain kits from providers offering technical support and useful online tools to help make label decisions easier and more straightforward.

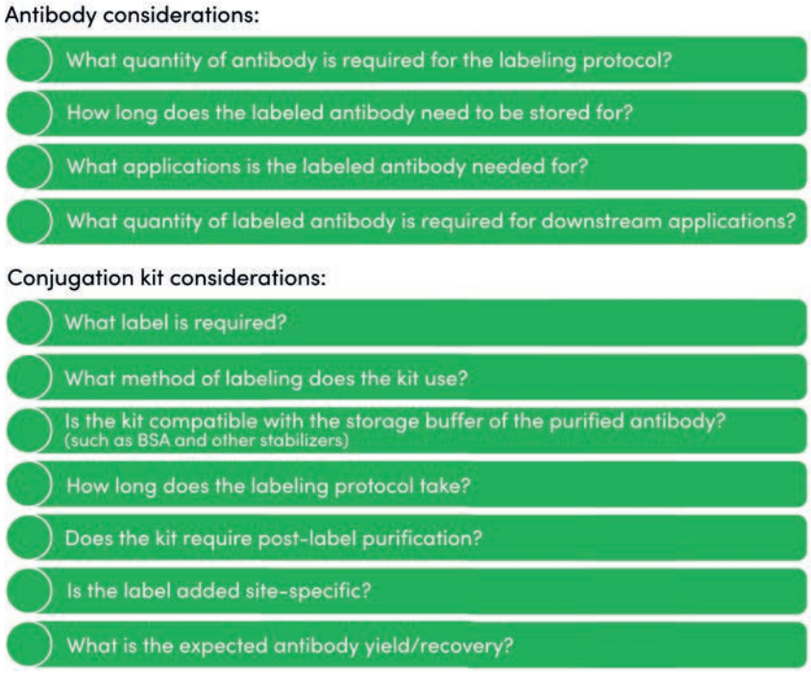

Figure 1: What researchers need to consider when selecting an appropriate conjugation kit.

Summary

The antibody conjugation kit market is rapidly growing and is estimated to reach $3.2 billion USD by 2033, growing at a CAGR of 9.2% from 2026 to 2033 [4]. This rapid growth is due in part to the technological advancements in conjugation techniques and rising demand for easy and reliable solutions to expand antibody toolboxes for better immunoassay development. Practically, conjugation kits allow improvements to assay design and performance while expanding experimental capabilities, especially for multiplexing. They also fill critical gaps where prelabelled antibody availability is more limited, such as in veterinary and translational immunology, and in pharma, where researchers need access to scalable and reliable reagents for in-house labelling. With a broad range of conjugation kits now available to meet different workflows and applications, it is now easier than ever to design and conduct high-quality immunoassays across diverse research areas.

References

1. *Jax E, et al. Evaluating effects of AIV infection status on ducks using a flow cytometry-based differential blood count. Microbiol Spectr. 2023;11(4):e0435122. doi:10.1128/spectrum.04351-22*
2. *Lo Giudice A, et al. Establishment of primary cell cultures from canine oral melanomas via fine-needle aspiration: a novel tool for tumorigenesis and cancer progression studies. Animals. 2024;14(13):1948. https://doi.org/10.3390/ani14131948*
3. *Gawronska-Kozak B, et al. Dermal white adipose tissue (dWAT) is regulated by Foxn1 and Hif-1α during the early phase of skin wound healing. Int J Mol Sci. 2021;23(1):257. doi:10.3390/ijms23010257*
4. *Verified Market Reports. Global antibody conjugation kits market size by type (direct conjugation kits, indirect conjugation kits), by antibody type (monoclonal antibodies, polyclonal antibodies), by label type (fluorophores, enzymes), by application (diagnostics, therapeutics), by end user (pharmaceutical companies, biotechnology firms), by geographic scope and forecast. March 2025. Available at: https://www.verifiedmarketreports.com/product/antibody-conjugation-kits-market/ (accessed August 29, 2025)*

Author bio: Sharon Sanderson, PhD, is the Product Manager for flow reagents and a Flow Cytometry Applications Scientist at Bio-Rad. She obtained her PhD in cancer cell biology from the University of London and spent thirteen years at the University of Oxford conducting research in cancer and immunology fields. In 2017, Sharon joined Bio-Rad as an Applications Scientist, before taking up her current position in 2022.