abcam

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Recombinant RabMAb®: Reliable antibodies for reproducible results

Optimize once and maintain performance long-term

Summary

- Poorly characterized antibodies often fail to provide consistent, reproducible results, thereby wasting materials, researcher time, and money. Compared to traditional polyclonal and monoclonal antibodies, recombinant antibodies exhibit very little batch-to-batch variability, providing scientists with highly reproducible results.

- In this white paper, we will showcase the reproducibility of recombinant antibodies by assessing the batch-to-batch consistency of the recombinant RabMab anti-PD-L1 antibody (28-8) (ab205921) across various applications, including IHC, ICC, flow cytometry, and western blot.

- Our results demonstrate high reproducibility between different batches of the recombinant anti-PD-L1 antibody tested in four different applications (IHC, ICC, flow cytometry, and western blot).

- Batch-to-batch reproducibility of recombinant antibodies overcomes the need for extensive downstream standardization assays saving time, cost, and/or resources.

- Consistency in the batch performance of recombinant antibodies ensures the longevity and reproducibility of research results generated with these reagents.

Introduction: Overcoming issues with antibody reproducibility by using recombinant antibodies

The 'reproducibility crisis' has highlighted the fact that many life science experiments cannot be replicated and that the conclusions based on these studies may be unfounded, with antibodies identified as an essential part of the problem[1-7]. Across biomedical research, global annual losses from using poorly characterized antibodies have been estimated at $800 million, not counting wasted research time and resources as well as the impact of false conclusions[4].

Variability between antibody batches often causes issues with antibody reproducibility, with studies failing to achieve the same results with different antibody batches in the same experimental setup[1,8,9]. Any proper validation of antibodies must therefore include evidence of robustness from batch to batch. However, validation of commercially available antibodies is not standardized and depends on the vendor, and high variation exists in the quality of the documentation accompanying the batches[1].

The method of antibody production influences antibody performance in terms of batch consistency (Table 1). Since polyclonal antibodies are derived from multiple B-cells of the serum of an immunized animal, they contain a heterogeneous mixture of antibodies, recognizing different epitopes on the same antigen, and are therefore prone to high batch-to-batch variability. Compared to polyclonals, monoclonal antibodies produced by hybridoma technology exhibit improved consistency between batches; however, potential genetic drift in hybridoma cell lines can lead to the irreproducibility of different antibody batches. Furthermore, 30% of hybridoma-produced monoclonal antibodies were shown to express additional productive heavy or light chains, rendering these antibodies non-specific[10].

Without reproducible reagents, project timelines may be missed due to extensive batch-to-batch standardization procedures or the requirement for new reagents to be developed mid-project. To overcome this reproducibility issue, save millions in research and development costs, and help improve the translation of research to the clinic, affinity reagents must be defined by their sequences and produced as recombinant proteins[4].

Recombinant antibodies are developed from a unique set of genes, allowing for controlled and reliable antibody production. Due to their production method, recombinant antibodies overcome batch-to-batch consistency issues of traditional polyclonal and monoclonal antibodies, including limitations of hybridoma-production of monoclonals such as gene loss, gene mutations, and cell-line drift. Engineered using leading-edge technologies to deliver high affinity and specificity, recombinant antibodies allow the use of reduced antibody concentrations compared to conventional antibodies.

	Polyclonal	Monoclonal	Recombinant
Production method	A heterogeneous mix of antibodies is derived from the immune response of multiple B-cells following animal immunization with the antigen of interest.	An antibody-producing B-cell from an immunized animal is fused with a myeloma cell to produce an antibody-producing hybridoma line.	The genetic sequence of the selected antibody is cloned into an expression vector, which is subsequently introduced into expression hosts to provide animal-free antibody production.
Reproducibility characteristics	Consists of multiple antibodies to various antigen epitopes. Following numerous rounds of serum collection, the animal is dispatched and the antibody source no longer available.	A single antibody clone, providing improved batch-to-batch consistency compared to polyclonals. Potential cell-line drift and mutations can impair production and result in antibody changes that affect reproducibility.	Stable production that can be re-generated at any time through the known genetic sequence starting material.

Table 1. Comparison between polyclonal, monoclonal, and recombinant antibodies in terms of production method and reproducibility.

To provide you with highly specific and reproducible antibodies, we have developed a large portfolio of recombinant versions of our RabMAb rabbit monoclonal antibodies (Fig. 1), with approximately 1000 new antibodies being launched annually to relevant targets across all applications. We are continuously working hard to improve recognition of the importance of recombinant antibodies in research and help you achieve reproducible results with an uninterrupted supply of antibodies.

Our recombinant RabMabs antibodies are fully validated for the selected applications listed on their datasheets, and we standardize each aliquot to perform according to the optimized protocols. Validation processes are built to ensure we repeatedly deliver reagents that show the same performance time after time, regardless of the scale of your project.

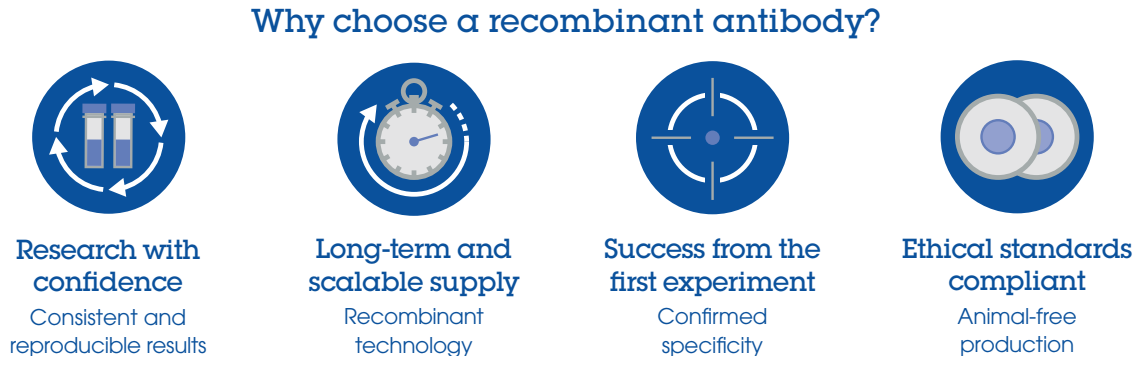


Figure 1. Summary of recombinant antibody features.

Results: Assessing batch-to-batch reproducibility of recombinant Anti-PD-L1 antibody in different applications

Immunohistochemistry

Across five independently manufactured batches of RabMAb Anti-PD-L1 antibody (28-8), the observed immunohistochemistry (IHC) results on formalin-fixed paraffin-embedded (FFPE) tissue demonstrate excellent consistency with reproducible, specific staining between batches.

Recombinant RabMAb® Anti-PD-L1 antibody [28-8] (ab205921) on FFPE human placenta

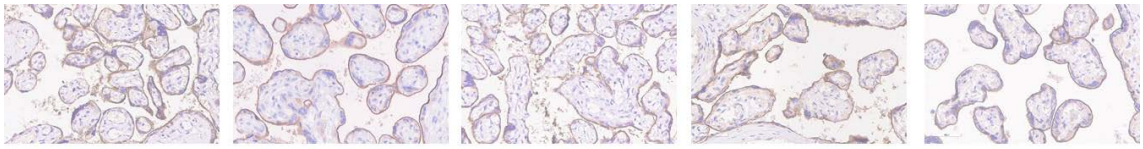


Figure 2. Batch-to-batch reproducibility in the localization of PD-L1 in the FFPE human placenta across five batches of RabMAb Anti-PD-L1 antibody (28-8) (ab205921). IHC performed manually using a Biocare Decloaking Device and Universal HIER antigen retrieval reagent (ab208572). The primary antibody was incubated overnight at 4°C, followed by Goat anti-rabbit secondary antibody and HRP-linked anti-goat polymer antibody (ab209101). DAB was used as a chromogen with a Hematoxylin counterstain.

Immunocytochemistry

Four separate batches of RabMAb Anti-PD-L1 antibody (28-8) were used to localize PD-L1 expression in CHO-S cells stably transfected with the immune checkpoint target. Immunolabeling with each of the different batches of antibody identified the same predicted membranous staining pattern of equal intensity in CHO-PD-L1 with negative staining in CHO-S.

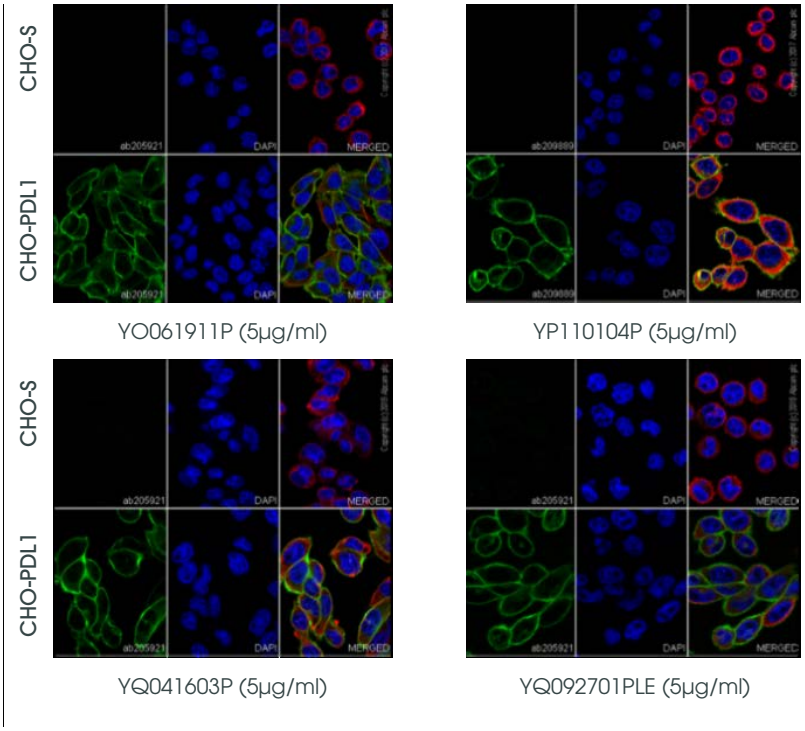


Figure 3. Reproducible immunocytochemistry labeling across four batches of RabMAb Anti-PD-L1 antibody (28-8) (ab205921). CHO-S and CHO-S stably transfected with PD-L1 were fixed in 4% PFA, permeabilized with 0.1% Triton X-100 and blocked with goat serum. The primary antibody was incubated overnight at 4°C, followed by Alexa Fluor® 488-conjugated Goat anti-Rabbit secondary antibody (ab150077) at 1/1000 dilution at RT for 30min. Alexa Fluor® 594 Anti-alpha Tubulin antibody (DM1A) (Ab195889) was used as a counterstain at 1/200 dilution at RT for 30min. Nuclei were visualized using DAPI.

Flow cytometry

Four batches of RabMAb Anti-PD-L1 antibody (28-8) were used to label PD-L1 in stably transfected CHO-S cells. Flow cytometric analysis showed consistent signal intensity across replicate antibody batches, with identical, distinct peaks delineating positive staining in viable CHO-PD-L1 cells and negative staining in viable null CHO-S cells.

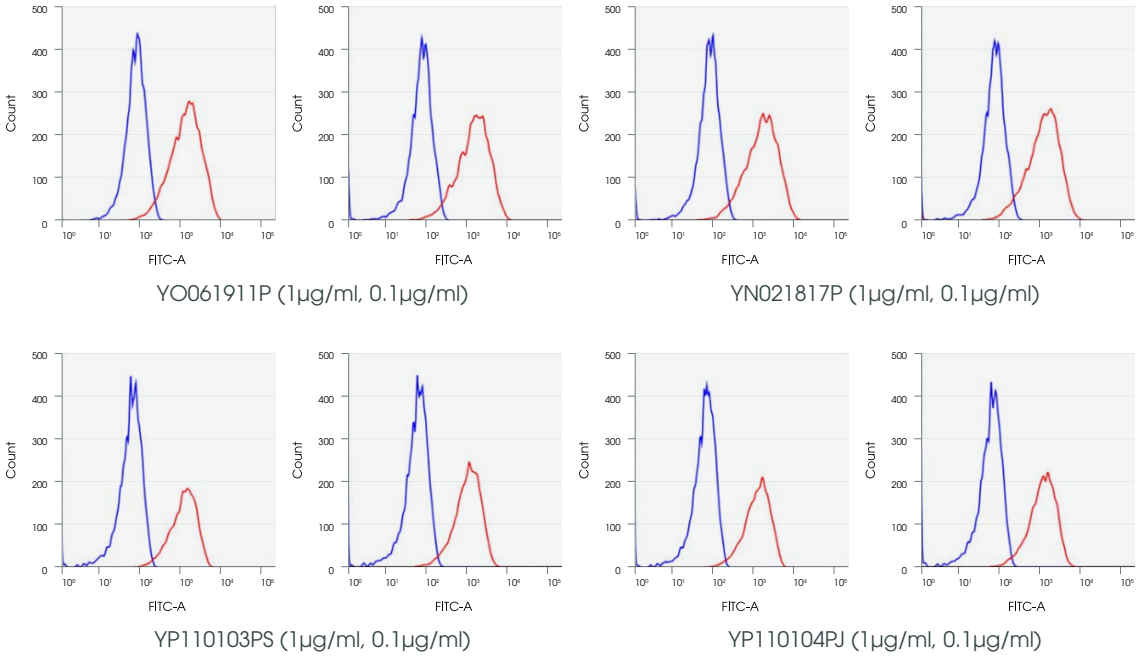
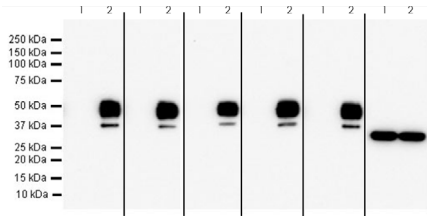


Figure 4. Batch-to-batch consistency in flow cytometric analysis across four batches of RabMAb Anti-PD-L1 antibody (28-8) (ab205921). CHO-S (blue line) and CHO stably transfected with PD-L1 cells (red line) were blocked with 10% goat serum for 1h at 4°C. The primary antibody was incubated for 30 min at 4°C, followed by Alexa Fluor® 488-conjugated goat anti-rabbit secondary antibody (ab150077) at 1/2000 dilution at 4°C for 30min. 7-AAD was incubated for 5min at 4°C prior to data acquisition via flow cytometer.

Western blot

Five independent antibody batches reveal equivalent, specific bands for the PD-L1 both in CHO-PD-L1 and U-87 MG cells via western blot. No non-specific signal or cross-reactive bands were observed, displaying a high level of batch-to-batch consistency across all assayed batches of recombinant antibody.

Recombinant Anti-PD-L1 antibody [28-8] (ab205921) in CHO-S and CHO-PD-L1 cells



Samples:
Lane 1: CHO-S (Chinese hampster ovary epithelial cell) whole cell lysates 15 µg
Lane 2: CHO-PD-L1 (PD-L1 stably expressed Chinese hampster ovary epithelial cell) whole cell lysates 15 µg

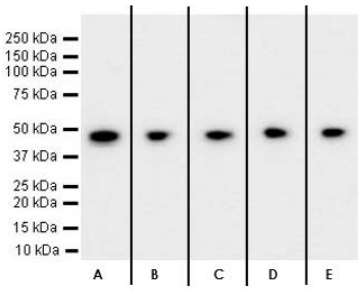
Primary antibodies:
A: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YN061622P produced in 2016)
B: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YP111536PDS produced in 2018)
C: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YP110104PJ produced in 2018)
D: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YQ092701PLE produced in 2019)
E: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YR051315PLE produced in 2020)
F: Anti-GAPDH antibody (EPR16891) (ab181602)

Secondary antibody:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051)(1:20.000 dilution)

Exposure time: 80 seconds

Figure 5. Batch-to-batch reproducibility in western blot detection of PD-L1 across five batches of RabMAb Anti-PD-L1 antibody (ab205921) in CHO-PD-L1 cells. CHO-S and CHO-PD-L1 cells were washed with cold 1 x PBS prior to rotating at 4°C for 45 min in RIPA lysis buffer. Cells were sonicated for 3 secs for 5–15 times with intervals of 10 secs at 40 kW until clear. Lysates were subject to SDS-PAGE at 150V for 1 h before transferring to PVDF. Blotting was performed manually using goat anti-rabbit IgG H&L (HRP) secondary antibody (ab97051) at 1:20,000 dilution for 1 h at RT prior to developing with ECL.

Recombinant Anti-PD-L1 antibody [28-8] (ab205921) in U-87 MG cells



Samples:
All lanes: U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysates 15 µg

Primary antibodies:
A: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YN061622P produced in 2016)
B: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YP111536PDS produced in 2018)
C: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YP110104PJ produced in 2018)
D: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YQ092701PLE produced in 2019)
E: Anti-PD-L1 antibody (28-8) (ab205921) at 1.0 µg/ml (YR051315PLE produced in 2020)

Secondary antibody:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051)(1:20.000 dilution)

Exposure time: 180 seconds

Figure 6. Batch-to-batch reproducibility in western blot detection of PD-L1 across five batches of RabMAb Anti-PD-L1 antibody (ab205921) in U-87 MG cells. U-87 MG cells were washed with cold 1 x PBS prior to incubating on ice for 15 min in RIPA lysis buffer. Cells were sonicated for 3 secs for 5–15 times with intervals of 10 secs at 40 kW until clear. Lysates were subject to SDS-PAGE at 150V for 1 h before transferring to PVDF. Blotting was performed manually using goat anti-rabbit IgG H&L (HRP) secondary antibody (ab97051) at 1:20,000 dilution for 1 h at RT prior to developing with ECL.

Summary: Leading the way forward with reproducible antibodies

The demand for sequence-defined recombinant antibodies continues to grow, particularly as part of various solutions to address issues associated with data reproducibility within scientific research[3-5]. While many researchers have adopted the use of monoclonal antibodies for increased target specificity[11], transitioning to the use of recombinant reagents is essential to increase the reproducibility and reliability of results within the life sciences[3-6].

Recombinant antibody production removes the variability associated with different antibody production batches and thus allows for the generation of reproducible results for research longevity. This long-term assurance of recombinant reagent performance could aid in the translation of research discoveries to diagnostic or drug development pipelines while protecting against any potential delays in time or cost, arising due to batch-to-batch reproducibility.

To learn more:

Recombinant antibodies: reproducible with tailored specificity

RabMAb® rabbit monoclonal antibodies: the advantages

Raising Antibody Standards white paper

Introduction to RabMAb primary antibody technology webinar

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