Antibodies

Assessing quality and performance: stability testing of antibodies shipped at ambient temperatures

Abstract

This study focuses on the stability of research-use antibodies supplied in various formats, buffers, and preservatives. The stability was assessed across time and under various temperature stressors. Using a statistically significant product sample set, we demonstrate a 100% pass rate based on set criteria and validate that this wide range of products can be shipped and maintained at ambient conditions for up to 7.5 days.

Introduction

To help mitigate elements of the environmental impact of our supply chain, Thermo Fisher Scientific recognized the urgent need to evaluate and update, where possible, our shipping process for Invitrogen™ antibodies. We set out to transition from a cold-chain packaging method to ambient shipping. This shift addresses environmental concerns and optimizes logistical efficiency. By transitioning antibody shipments to ambient conditions, the packaging weight is reduced, resulting in a decrease in carbon emissions during transportation.

Historically, Invitrogen antibodies have been shipped utilizing cold-chain packaging to maintain their quality and integrity during transit. As such, any updates to our shipping process must be accompanied by confirmation that the quality of the product is unaffected.

The purpose of this study was to investigate antibody performance after being exposed to a heat-stressed environment (e.g., 37°C). The impact of heat stress was assessed using established quality specifications in chosen functional tests. For each antibody, functional testing was performed by one or more of the following assays: western blot, immunohistochemistry (IHC), immunofluorescence (IF), immunocytochemistry (ICC), enzyme-linked immunosorbent assay (ELISA), dot blot, and sample normalization and antibody profiling for chromatin immunoprecipitation (SNAP-ChIP™ assay). Our validation procedure comprised a subset of antibodies selected to help ensure robust statistical significance and to be representative of our comprehensive antibody portfolio.

The validation process yielded compelling results, affirming the feasibility and effectiveness of the ambient-temperature shipping process for antibodies regardless of the format (buffers, stabilizers, preservatives, and conjugates). Extensive testing revealed that the quality and shelf life of the product remained uncompromised, confirming our hypothesis and supporting the new shipping method. These findings underscore the potential of using ambient-temperature shipping as a more sustainable alternative without sacrificing antibody functionality.

Validation methodology

Our portfolio of Invitrogen antibodies has over 233,000 catalog products that include primary and secondary antibodies with conjugates from various dye classes and isotype controls. The majority of these products are currently packaged in a variety of boxes, some including expanded polystyrene (EPS) coolers with multiple ice packs, and shipped to the customer.

For the validation plan, we analyzed our antibody catalog and identified 5 subsets of products (Table 1), representing 80% of the portfolio. The rationale behind creating these subsets was to ensure coverage for a wide variety of buffers, stabilizers, preservatives, and conjugates. The minimum number of samples to test in each subset was determined using a statistical approach that is employed in quality control (QC) inspections (Table 2).

To understand the effects of temperature on antibody functionality in a stressed environment, we created a stability test using an industry-accepted criterion for accelerated stability studies. We developed our validation conditions to simulate a scenario where an antibody was in transit for 2 days at 37°C (considered a stressed condition) with an additional 5.5 days of exposure to room temperature (20–25°C). As we assume that some shipments could be exposed to conditions of extreme heat, we also included a shock treatment of 4–8 hours at 45°C to simulate that extreme heat for short periods of the day. In the real world, this test translates to a duration of up to 7.5 days from the time an antibody package shipped at room temperature leaves our distribution center and reaches a customer's lab. Acceptance criteria for the test samples are specified in Table 3.

Table 1. Subsets of Invitrogen antibodies used in the study.

Subset	Preservative	Buffer	Additive	Conjugate
1	Any sodium azide percentage	Any PBS formulation	No additive	None
2	Any sodium azide percentage	Any PBS formulation	BSA	None
3	No preservative	Any buffer	Any additive	None
4	0.016% methylisothiazolone, 0.16% bromonitrodioxane or Kathon™ microbicide	Proprietary buffer, any buffer	Any additive	Invitrogen™ Alexa Fluor™ Plus and HRP conjugates
5	Any preservative	Any buffer	Any additive	Yes, includes major dye classes

Table 2. Number of Invitrogen antibodies (Abs) in each subset, and minimum number of samples to test per subset.

Subset	Abs in subset	Minimum number of samples required*
1	16,611	57
2	40,858	60
3	38,494	57
4	184	22
5	29,201	38

* Determined using a zero-acceptance number sampling plan [1]. In this approach, the minimum numbers of samples to test are based on statistical calculations that account for the overall sample size and desired level of confidence. This plan was selected for its stringency of QC—for a subset to pass, no failures from the selected minimum sample size are acceptable.

Table 3. Summary of acceptance criteria.

Number	Key parameter	Acceptance criterion
1	Release specifications	The test sample undergoing the stability study was tested along with a control sample, following the established QC specifications.
2	Product performance in customer applications	For all antibodies, the test sample (undergoing the stability study) shows equivalent minimum performance in the selected application relative to the control sample. **Note:** Results are considered equivalent if the functional characteristics (e.g., signal, band intensity, target specificity) are deemed similar, and the difference between samples does not exceed normal anticipated assay variation.

Materials and methods

Materials

The antibodies used in this stability study were sourced from various Thermo Fisher Scientific distribution centers and suppliers. They were pulled from current or previously sold inventory that had already passed QC tests and been released into the market. When available, multiple lots of an antibody produced previously were included to determine lot-to-lot consistency.

The remaining testing materials used in this study included SDS-PAGE gels, electrophoresis buffers, chemiluminescent and colorimetric substrates, lysates, tissues, isotype controls, flow cytometry reagents, and secondary antibodies. These materials were freshly purchased or from in-house supply at various Thermo Fisher Scientific antibody manufacturing and testing laboratories.

Stability study

The stability study and the functional tests were performed at various Thermo Fisher Scientific sites around the world. To avoid operator bias, multiple scientists were involved in the testing process. Selected antibodies (multiple lots when available, 1 or 2 vials per lot) from each subset were collected and placed in their normal storage temperature location (–20°C or 2–8°C). An antibody vial of each lot was thawed (where applicable) and split into two separate vials, and labeled as control and test along with other product details.

The control group samples were returned to their normal storage temperature location (–20°C or 2–8°C). To simulate extended exposure to fluctuations during room temperature conditions, the test group samples were placed in an incubator set to 37°C for a minimum of 4 days and then moved to another incubator set to 45°C for 4–8 hours. After both incubations, the test group samples were returned to their normal storage temperature location (–20°C or 2–8°C) and stored until ready for testing.

Western blot

In western blot analysis, based on the QC test specifications, a protein sample (from tissues, cells, overexpressed proteins, or recombinant proteins) was separated based on size through gel electrophoresis. The separated proteins were then transferred to a membrane (nitrocellulose or PVDF). This membrane was probed with antibody samples from the stability study that bind to the target protein of interest, followed by a secondary antibody (where applicable). Finally, the bound antibodies were visualized using a chemiluminescence or fluorescence detection system (Invitrogen™ iBright™ system or equivalent).

Dot blot

In dot blot analysis, based on the QC test specifications, a pure protein was blotted onto a membrane (nitrocellulose or PVDF). This membrane was probed with antibody samples from the stability study that bind to the pure protein, followed by a secondary antibody (where applicable). The bound antibodies were then visualized using a chemiluminescence detection system (iBright system or equivalent).

ELISA

In ELISA, based on the QC test specifications, a target molecule (antigen, protein, or antibody isotype control) and nontarget molecules (where applicable) were immobilized in a 96-well plate. Antibody samples from the stability study were used to detect the target, and when necessary an HRP- or fluorophore-conjugated secondary antibody was added. To detect and measure the colorimetric, chemiluminescent, or fluorescent signal, an appropriate plate reader was used. If the test required a cross-reactivity specification, nontarget molecules were included in the test.

IHC

In IHC, based on the QC test specifications, tissue samples (frozen or paraffin-embedded and fixed) were probed with antibody samples from the stability study. When necessary, an HRP-conjugated secondary antibody was added. An appropriate colorimetric detection method (e.g., DAB, AEC) and a microscope were used to visualize staining of the target protein.

IF

In IF, based on the QC test specifications, fixed cells or tissue samples (frozen or paraffin-embedded and fixed) were probed with antibody samples from the stability study. When necessary, a fluorophore-conjugated secondary antibody was added. A fluorescence imaging microscope (Invitrogen™ EVOS™ imaging system or equivalent) was used to visualize staining of the target protein.

Flow cytometry

In flow cytometry, based on the QC test specifications, antibody samples from the stability study were used to stain target markers on single cells (e.g., peripheral blood cells, mouse spleen cells, cell lines). When necessary, a fluorophore-conjugated secondary antibody was added. Fluorescent signal intensities and patterns of the samples were acquired by a flow cytometer (Invitrogen™ Attune™ flow cytometer or equivalent) equipped with appropriate excitation and emission filters.

SNAP-ChIP assay

In the SNAP-ChIP assay, based on the QC test specifications, antibody samples from the stability study were used to determine if the antibody was pulling down the intended histone modification from isolated DNA of appropriate cells. Additionally, antibody pulldown of any other (nonspecific) modification among a panel of histone posttranslational modifications (PTMs), distinguished based on their DNA barcodes, was analyzed.

Results

From 5 subsets, a total of 192 unique antibodies representing 311 different lots were tested. Based on the established quality specifications, 100% of the test and control antibodies passed stability testing after a 37°C incubation for 4 days, followed by temperature shock at 45°C for 4–8 hours (Table 4).

Figures 1–10 show example results from some of our most popular antibodies. When stressed in conditions (37°C to 45°C) well above an antibody's normal storage temperature range (–20°C to 4°C), Invitrogen antibodies perform similar to the control sample that was not stressed.

Table 4. Summary of stability testing results.

Subset	Individual Abs tested	Ab samples tested (including multiple lots)	Pass rate
1	54	107	100%
2	37	70	100%
3	47	77	100%
4	21	55	100%
5	33	36	100%

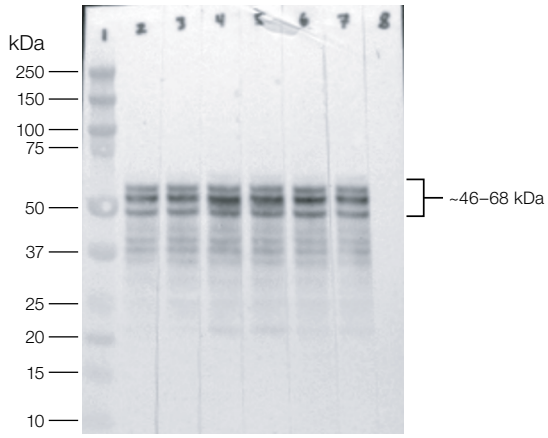

Figure 1. Tau antibody stability test in western blot. Western blot analysis was performed using test and control samples of Invitrogen™ Tau Monoclonal Antibody (TAU-5) (Cat. No. AHB0042) from 3 different lots. Bands at ~46–68 kDa corresponding to tau were observed in human brain lysates using both test and control samples (lane 1: molecular weight marker; lanes 2, 4, and 6: control samples of lots A, B, and C; lanes 3, 5, and 7: test samples of lots A, B, and C; lane 8: negative control). The lysate (20 µg lysate/lane) was electrophoresed in an Invitrogen™ NuPAGE™ 4–12% Bis-Tris Protein Gel (Cat. No. NP0322BOX). Resolved proteins were then transferred to a Thermo Scientific™ nitrocellulose membrane (Cat. No. 77012). The blot was probed with the primary antibody (2 µg/mL) and detected by chemiluminescence with Invitrogen™ Goat Anti–Mouse IgG (H+L) Secondary Antibody, HRP (Cat. No. 31430, 1:50,000) and the Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate (Cat. No. 34076), using the Invitrogen™ iBright™ FL1000 Imaging System.

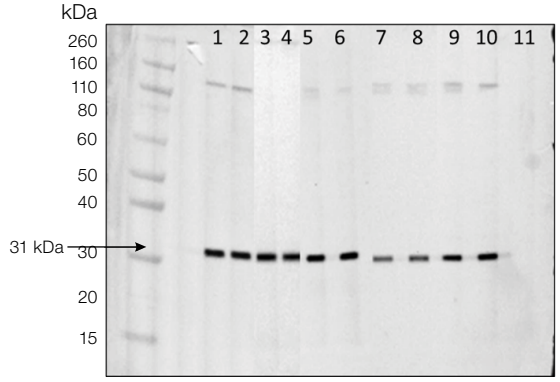

Figure 2. Phospho-S6 (Ser244, Ser247) antibody stability test in western blot. Western blot analysis was performed using test and control samples of Invitrogen™ Anti–Phospho-S6 (Ser244, Ser247) Polyclonal Antibody (Cat. No. 44-923G) from 5 different lots. A band at ~31 kDa corresponding to phospho-S6 (Ser244, Ser247) was observed in TNFα-treated HeLa lysates using both test and control samples (lanes 2, 4, 6, 8, and 10: control samples of lots A, B, C, D, and E; lanes 1, 3, 5, 7, and 9: test samples of lots A, B, C, D, and E; lane 11: negative control). Lysates of TNFα-treated HeLa cells (15 µg lysate/lane) were electrophoresed in a NuPAGE 4–12% Bis-Tris Protein Gel (Cat. No. NP0326BOX). Resolved proteins were then transferred to a nitrocellulose membrane. The blot was probed with the primary antibody (1 µg/mL) and detected by chemiluminescence with Invitrogen™ Goat Anti–Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP (Cat. No. G21234, 1:10,000), using the iBright FL1000 Imaging System. Chemiluminescent detection was performed using the Invitrogen™ Novex™ ECL Chemiluminescent Substrate Reagent Kit (Cat. No. WP20005).

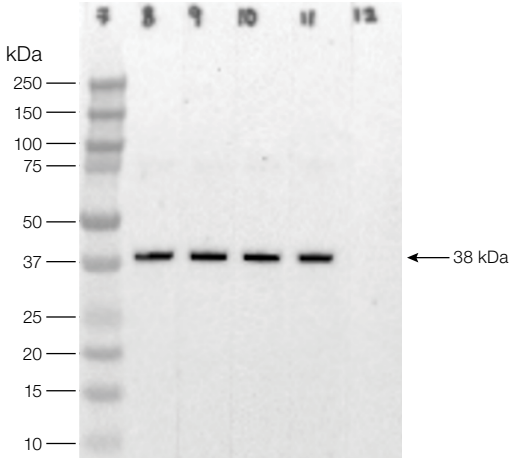

Figure 3. HIF1A antibody stability test in western blot. Western blot analysis was performed using test and control samples of Invitrogen™ HIF1A Monoclonal Antibody (mgc3) (Cat. No. MA1-516) from 2 different lots. A band at ~38 kDa corresponding to recombinant human HIF1A protein was observed using both test and control samples (lane 7: molecular weight marker; lanes 8 and 10: control samples of lots A and B; lanes 9 and 11: test samples of lots A and B; lane 12: negative control). A lysate containing recombinant human HIF1A protein (2.5 ng lysate/lane) was electrophoresed in a NuPAGE 4–12% Bis-Tris Protein Gel (Cat. No. NP0322BOX). Resolved proteins were then transferred to a nitrocellulose membrane (Cat. No. 77012). The blot was probed with the primary antibody (1 µg/mL) and detected by chemiluminescence with Goat Anti–Mouse IgG (H+L) Secondary Antibody, HRP (Cat. No. 31430,1:50,000) and the SuperSignal West Dura Extended Duration Substrate (Cat. No. 34076), using the iBright FL1000 Imaging System.

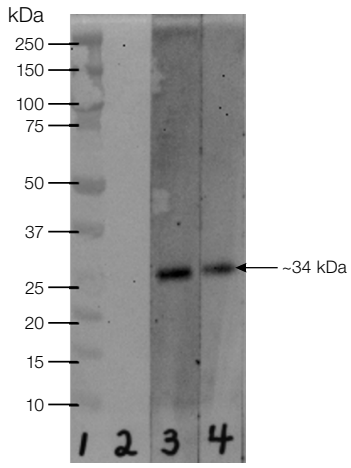

Figure 4. GFP antibody stability test in western blot. Western blot analysis was performed using test and control samples of Invitrogen™ GFP Polyclonal Antibody (Cat. No. A-11122). A band at ~34 kDa corresponding to recombinant GFP-tagged protein was observed using both test and control samples (lane 1: molecular weight marker; lane 2: negative control; lane 3: control sample; lane 4: test sample). A lysate containing recombinant GFP-tagged protein (500 ng lysate/lane) was electrophoresed in a NuPAGE 4–12% Bis-Tris Protein Gel (Cat. No. NP0322BOX). Resolved proteins were then transferred to a nitrocellulose membrane (Cat. No. 77012). The blot was probed with the primary antibody (2 µg/mL) and detected by chemiluminescence with Invitrogen™ Donkey Anti–Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP (Cat. No. 31458, 1:50,000) and the SuperSignal West Dura Extended Duration Substrate (Cat. No. 34076), using the iBright FL1000 Imaging System.

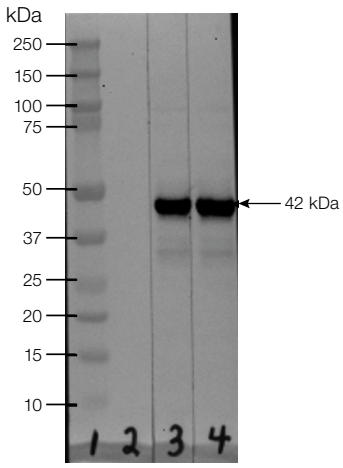

Figure 5. Alpha smooth muscle actin antibody stability test in western blot. Western blot analysis was performed using test and control samples of Invitrogen™ eBioscience™ Alpha Smooth Muscle Actin Monoclonal Antibody (1A4) (Cat. No. 14-9760-82). A band at ~42 kDa corresponding to alpha smooth muscle actin was observed in human heart tissue extract using both test and control samples (lane 1: molecular weight marker; lane 2: negative control; lane 3: control sample; lane 4: test sample). The human heart tissue extract (30 µg lysate/lane) was electrophoresed in a NuPAGE 4–12% Bis-Tris Protein Gel (Cat. No. NP0322BOX). Resolved proteins were then transferred to a nitrocellulose membrane (Cat. No. 77012). The blot was probed with the primary antibody (1 µg/mL) and detected by chemiluminescence with Goat Anti–Mouse IgG (H+L) Secondary Antibody, HRP (Cat. No. 31430, 1:50,000) and the SuperSignal West Dura Extended Duration Substrate (Cat. No. 34076), using the iBright FL1000 Imaging System.

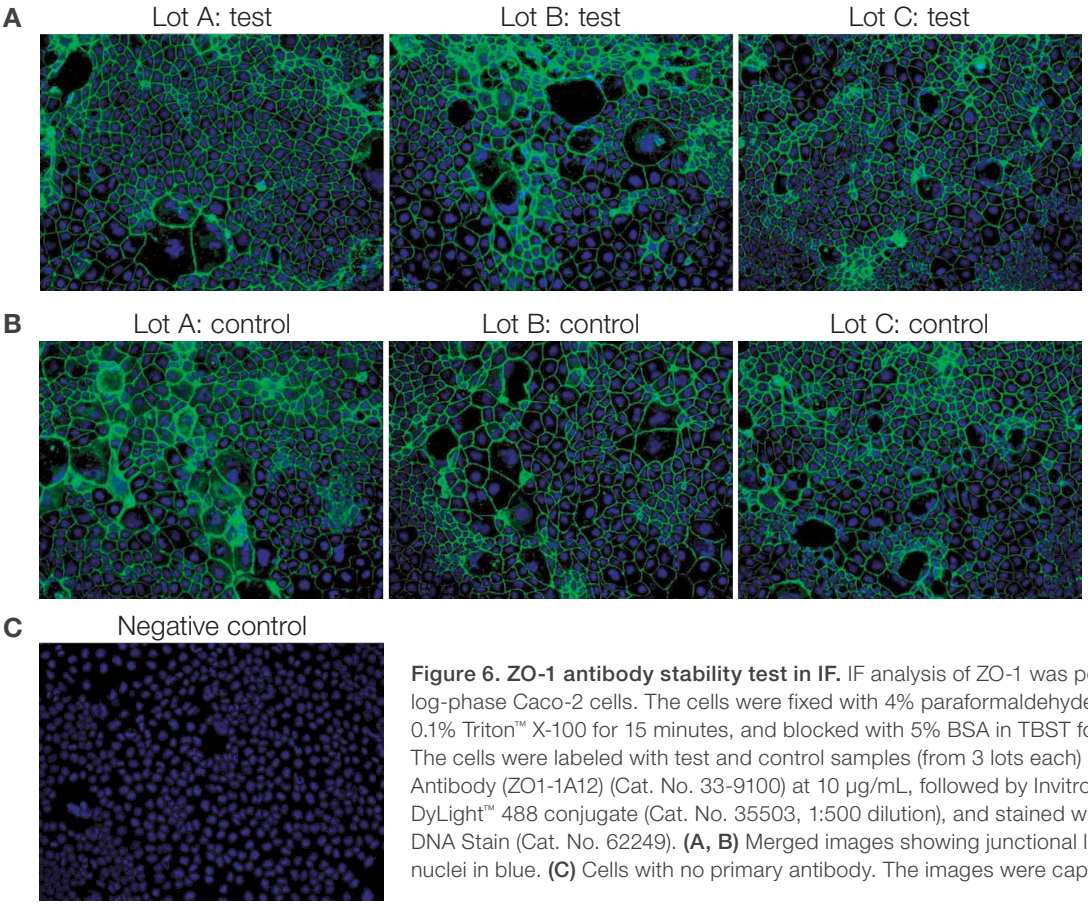

Figure 6. ZO-1 antibody stability test in IF. IF analysis of ZO-1 was performed using 90% confluent log-phase Caco-2 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 5% BSA in TBST for 45 minutes at room temperature. The cells were labeled with test and control samples (from 3 lots each) of Invitrogen™ ZO-1 Monoclonal Antibody (ZO1-1A12) (Cat. No. 33-9100) at 10 µg/mL, followed by Invitrogen™ Goat Anti–Mouse IgG, DyLight™ 488 conjugate (Cat. No. 35503, 1:500 dilution), and stained with Thermo Scientific™ Hoechst™ DNA Stain (Cat. No. 62249). **(A, B)** Merged images showing junctional localization of ZO-1 in green and nuclei in blue. **(C)** Cells with no primary antibody. The images were captured at 10x magnification.

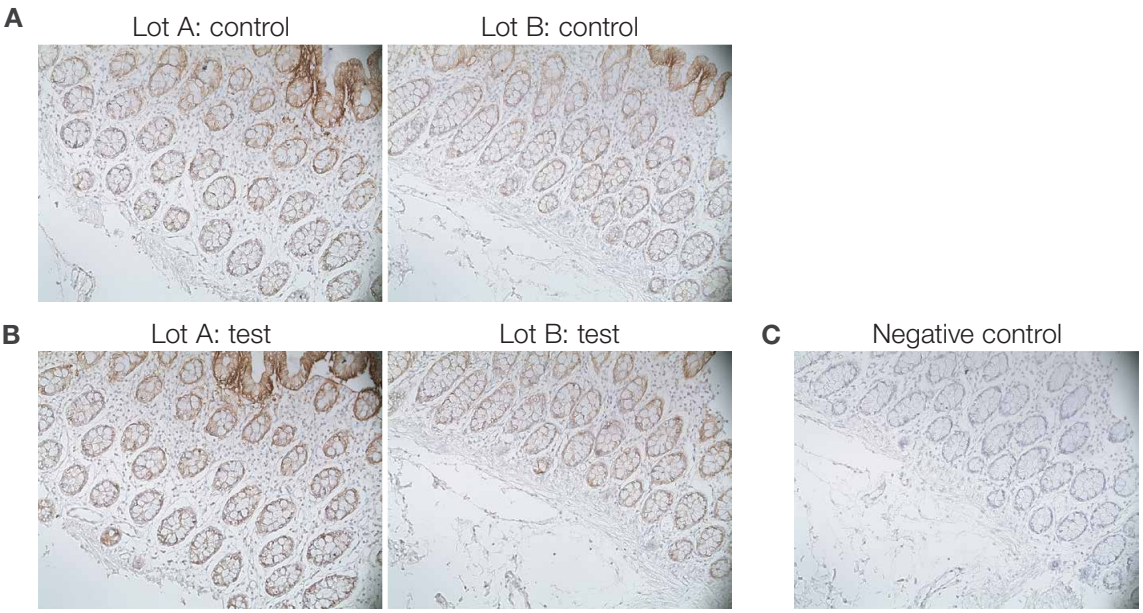

Figure 7. Claudin 4 antibody stability test in IHC. To expose target proteins, antigen retrieval was performed by using a dewax and heat-induced epitope retrieval (HIER) buffer and heating for 20 minutes in the Lab Vision™ PT Module. Following antigen retrieval, tissues were blocked in 3% hydrogen peroxide for 15 minutes, followed by 10% goat serum for 30 minutes, washed with 1X TBST, and then probed with 2 different lots of test and control samples of Invitrogen™ Claudin 4 Monoclonal Antibody (3E2C1) (Cat. No. 32-9400) diluted in 10% goat serum to 5 µg/mL, for 1 hour at room temperature. Tissues were washed extensively in 1X TBST, and colorimetric detection was performed with Goat Anti–Mouse IgG (H+L) Secondary Antibody, HRP (Cat. No. 31430, 1:500) and a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene before mounting. **(A, B)** Membrane localization of claudin 4 in human colon tissue. **(C)** Human colon tissue with no primary antibody. The images were captured at 20x magnification.

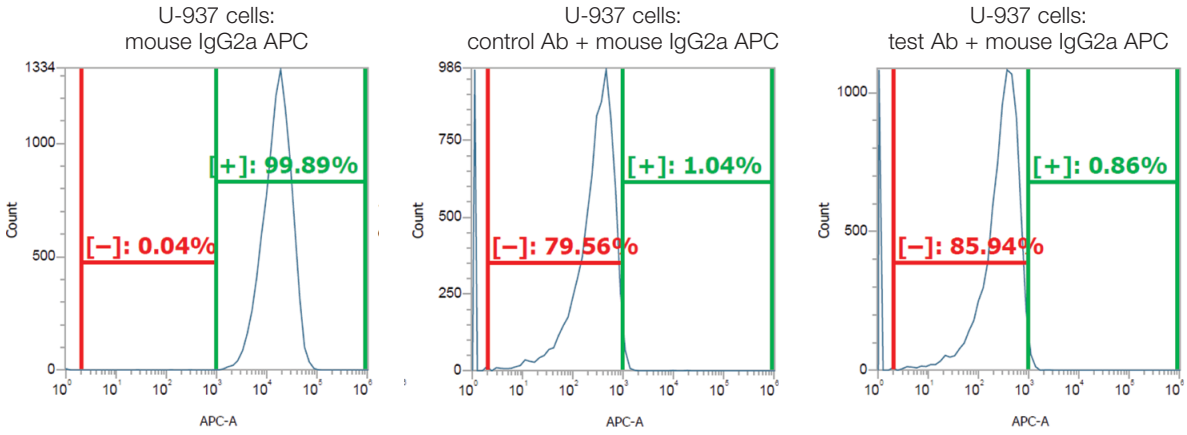

Figure 8. Fc receptor binding inhibitor antibody stability test in flow cytometry. U-937 cells were either left untreated or treated with Invitrogen™ Fc Receptor Binding Inhibitor Polyclonal Antibody (Cat. No. 16-9161-73; 40 µL/test). Left: untreated cells; middle: control sample; right: test sample). Cells were stained with Invitrogen™ eBioscience™ Mouse IgG2a Kappa Isotype Control (eBM2a), APC (Cat. No. 17-4724-81, 1:100). Flow cytometric analysis was performed on the Invitrogen™ Attune™ NxT Flow Cytometer.

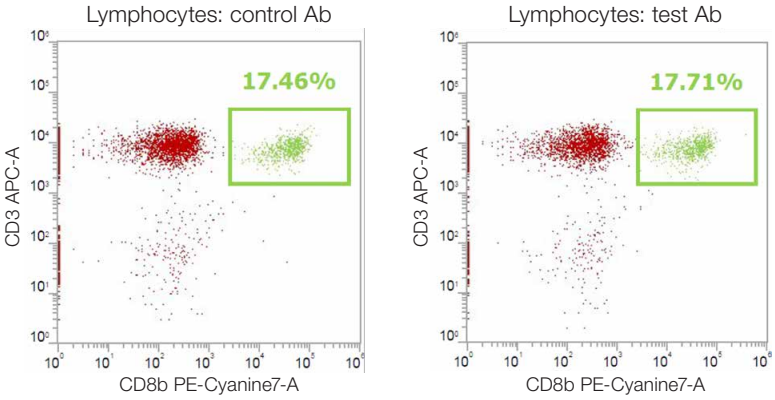

Figure 9. CD8b antibody stability test in flow cytometry. Normal human peripheral blood cells were stained with either control (left) or test (right) samples of Invitrogen™ CD8b Monoclonal Antibody (SIDI8BEE), PE-Cyanine7 (Cat. No. 25-5273-42; 1 µL/test) and co-stained with Invitrogen™ CD3 Monoclonal Antibody (SK7), APC (Cat. No. 17-0036-42). Cells in the lymphocyte gate were used for analysis. Flow cytometric analysis was performed on the Attune NxT Flow Cytometer.

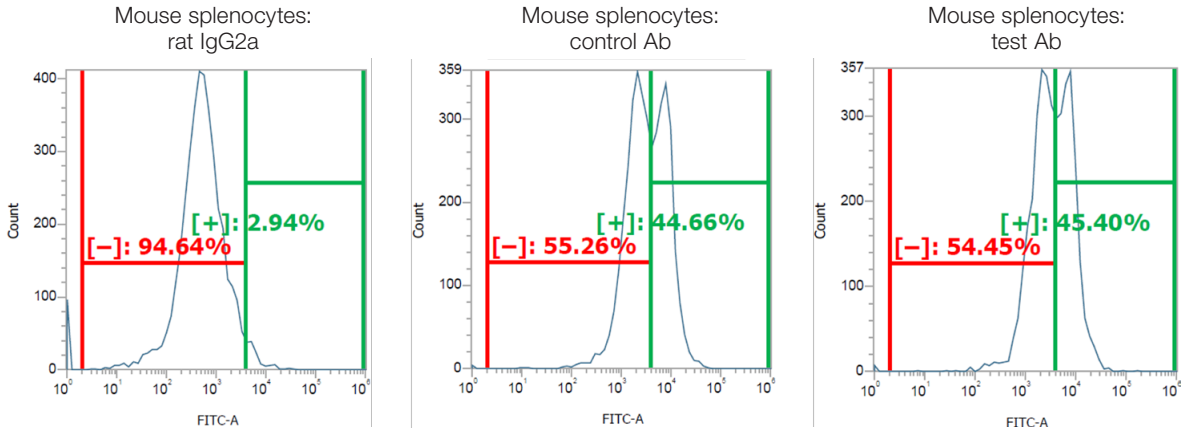

Figure 10. CD16/CD32 antibody stability test in flow cytometry. C57BL/6 mouse splenocytes were stained with either Invitrogen™ Rat IgG2a Isotype Control (Cat. No. 02-9688; 3 µL/test) (left), or control (middle) or test (right) samples of Invitrogen™ eBioscience™ CD16/CD32 Monoclonal Antibody (93) (Cat. No. 14-0161-85; 6 µL/test) and followed by Invitrogen™ Goat Anti–Rat IgG Secondary Antibody, FITC (Cat. No. 31629). Flow cytometric analysis was performed on the Attune NxT Flow Cytometer.

Conclusion

Through functional and stability testing, we have demonstrated that antibody products distributed under ambient-temperature shipping conditions perform identically to those shipped under cold-chain temperature conditions, and maintain long-term stability.

Reference

1. Squeglia NL (2008) *Zero acceptance number sampling plans.* Fifth edition. ASQ Quality Press.

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