

Novex® NuPAGE® Bis-Tris Electrophoresis System:

Performance comparison with the Mini-PROTEAN® TGX™ System

The Novex® NuPAGE® Bis-Tris Electrophoresis System uses a revolutionary neutral-pH precast polyacrylamide gel and unique buffers to provide unmatched band resolution and higher protein and gel stability compared to traditional Tris-glycine SDS-PAGE systems [1,2]. This application note compares the performance of the Novex® NuPAGE® Bis-Tris gel system to that of the Bio-Rad® Mini-PROTEAN® TGX™ (Tris-glycine extended) system. We compare the quality of protein separation and performance of the gels in western blotting, and the results demonstrate the superior performance of the Novex® NuPAGE® Bis-Tris Electrophoresis System.

Novex® NuPAGE® Bis-Tris gel system—the new gold standard in protein gel electrophoresis

The conventional Tris-glycine polyacrylamide gel system, as originally described by Laemmli [3], was once considered the gold standard for protein gel electrophoresis. However, while representing a significant improvement over previous technologies,

Laemmli Tris-glycine gels suffer from a number of drawbacks, including short useful shelf life (4–6 weeks), long run times (~90 minutes), band broadening, lane “smiling,” significant protein degradation and protein modification [4–8], and relatively low efficiency in protein blotting.

The Novex® NuPAGE® Bis-Tris polyacrylamide gel electrophoresis system was specifically developed to address the shortcomings of the Laemmli-type PAGE gels mentioned above, and has become the new gold standard in protein electrophoresis. Researchers have cited the use of Novex® NuPAGE® gels in over 5,000 publications, which is more than for any other ready-to-use PAGE gel available on the market.

The Novex® NuPAGE® Bis-Tris Electrophoresis System offers:

- Excellent resolution—due to improved protein separation
- Efficient transfer—due to low polyacrylamide concentration

- Fast run times—complete electrophoresis in as little as 35 minutes
- High gel stability—shelf life of >12 months at room temperature
- Tunable separation—achieve two different ranges of separation with the same gel, just by using different running buffers

As new precast gels become available on the market, their performance is often compared to that of the Novex® NuPAGE® Bis-Tris gel system. This application note compares the protein separation and western transfer performance of the Novex® NuPAGE® system to that of the Bio-Rad® Mini-PROTEAN® TGX™ System.

Results

Protein electrophoresis separation

The performance of the Novex® NuPAGE® Bis-Tris gel system and the TGX™ gel system was tested using a wide variety of samples and protein loads. The results were visually evaluated.

One of the most significant issues found with the TGX™ gel system, when prepared and run with Tris-glycine sample buffer as recommended by the manufacturer, was the marked sample degradation observed with high protein loads. The degradation was readily apparent when pure proteins such as lysozyme and BSA were run at higher loads (Figure 1, lanes 3 and 5).

In addition, the Mark12™ and MagicMark™ XP standard protein bands broadened at the bottom of the TGX™ gels. The same standards ran more uniformly on Novex® NuPAGE® gels (Figure 1, lanes 1 and 6). In the TGX™ gels, the high molecular weight protein bands (Figure 1B, lanes 1, 4, and 10) showed significant curving, which is consistent with results shown in Bio-Rad's own literature (Bulletin 5910, Bio-Rad Laboratories, Inc.).

Novex® NuPAGE® gels have a higher buffering capacity and are better at handling samples with different ionic strengths compared to TGX™ gels. As illustrated in Figure 1, this results in efficient separation of proteins without band broadening or narrowing.

The TGX™ gels also exhibit higher background when compared to Novex® NuPAGE® gels stained with SimplyBlue™ SafeStain (Figure 1).

Fast run protocols

We compared the quality of protein separation using accelerated electrophoresis protocols according to each manufacturer's recommendations, as described in the methods. When run under accelerated conditions, the TGX™ gels ran slightly faster but showed more band broadening and curvature (Figure 2B) compared to TGX™ gels run under standard conditions (Figure 1B). Novex® NuPAGE® gels run under accelerated conditions displayed sharp and straight bands with no compromise in performance (Figure 2A).

Protein blotting and immunodetection

The protein blotting efficiency of Novex® NuPAGE® and TGX™ gels was compared using both a standard wet blotting process and the iBlot® Dry Blotting System. Samples containing proteins of a wide range of sizes (*E. coli* lysate and MagicMark™ XP Western Standard) were separated on the gels, transferred, and then detected using an anti-*E. coli* primary antibody and the WesternBreeze® Chromogenic Kit. Each manufacturer's recommended procedure was used for the wet transfer protocol. We performed the iBlot® transfers using the standard P3 protocol (20 V for 7 min) for both types of gels.

Wet transfer

Use of the Novex® NuPAGE® 4–12% Bis-Tris gels and associated protocols resulted in more sensitive detection of the *E. coli* lysate and the MagicMark™ XP Western Protein Standard, compared to TGX™ gels (Figure 3, lane 7). More bands were seen on the membrane following transfer from Novex® NuPAGE® gels compared to transfer from the TGX™ gels. Additionally, western blots of TGX™ gels generally exhibited more smearing and higher background between the bands compared to blots from Novex® NuPAGE® gels. This phenomenon is demonstrated in Figure 3 (lanes 8–10 of gels A and B).

iBlot® transfer

When the iBlot® device was used for the blotting process, western blots of the Novex® NuPAGE® 4–12% Bis-Tris gels demonstrated more sensitive detection of the *E. coli* lysate and the MagicMark™ XP Western Protein Standard, compared to western blots of TGX™ gels (Figure 4).

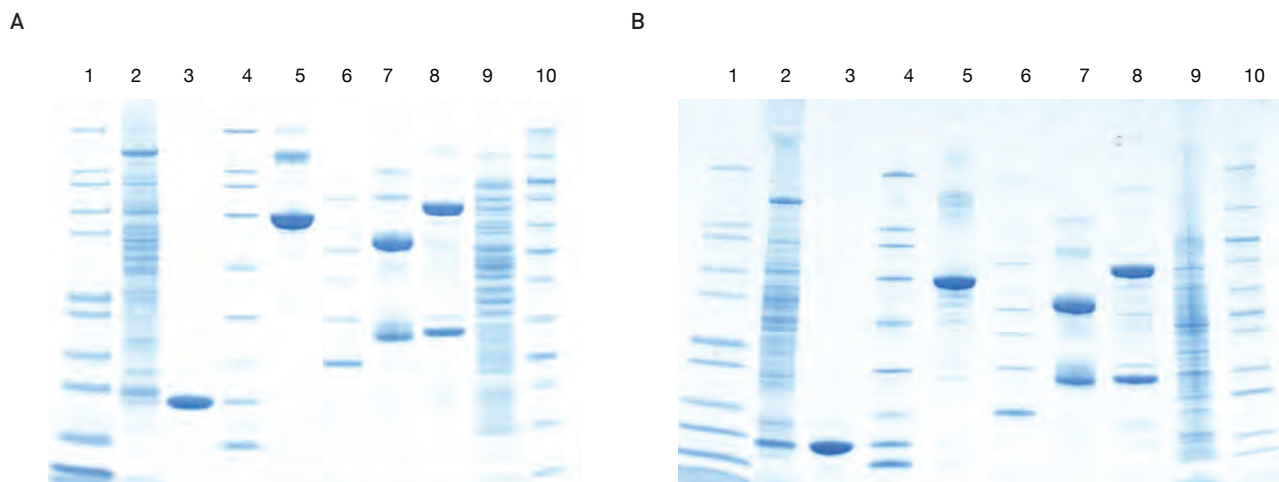


Figure 1. Superior protein electrophoresis separation using the Novex® NuPAGE® Bis-Tris gel system (A) compared to the Bio-Rad® TGX™ system (B). Samples loaded: (1) Mark12™ Unstained Standard, 10 μ L; (2) rat liver lysate, 10 μ g; (3) lysozyme, 6 μ g; (4) Bio-Rad® Broad Range Standard, 1:50 dilution; (5) BSA, 6 μ g; (6) MagicMark™ XP Standard, 10 μ L; (7) human IgG, 6 μ g; (8) human IgM, 6 μ g; (9) *E. coli* lysate, 10 μ g; (10) Novex® Sharp Unstained Standard, 10 μ L.

Long-term shelf life

Novex® NuPAGE® Bis-Tris gels are proven to be functional for one year when stored at room temperature. TGX™ gels are advertised to have one year of stability when stored at 4°C; however, TGX™ stability studies were conducted under accelerated conditions. To empirically evaluate the shelf life of both products, we compared the performance of Novex® NuPAGE® and TGX™ gels within one month of receipt and after 6 months of storage under the manufacturers' recommended conditions. The Novex® NuPAGE® gels demonstrated consistent separation and sharp protein bands after 6 months, while curvature of the protein bands was observed in the TGX™ gels (Figure 5).

Handling notes

TGX™ gels are more fragile compared to Novex® NuPAGE® gels. In our tests, many of the TGX™ gels broke during handling, making it a challenge to obtain intact gel images or membrane transfers for these comparisons.

Conclusion

Despite its popularity, the traditional Laemmli Tris-glycine protein gel electrophoresis system suffers from inherent problems, including poor buffering capacity during sample preparation and gel electrophoresis, protein modification, short shelf life, and low protein transfer efficiency. The Novex® NuPAGE® Bis-Tris gel system addresses these issues and enables superior protein separation and western blot detection.

The Bio-Rad® TGX™ gel system, while advertising long shelf life and faster run times, still suffers from some of the fundamental shortcomings of the Tris-glycine system, such as protein degradation during sample preparation. The TGX™ gel system uses Tris-glycine sample buffer prepared at pH 6.8. When samples are heated in this buffer, the pH decreases due to the negative temperature coefficient of Tris, resulting in protein hydrolysis as seen in Figure 1. The Novex® NuPAGE® system preserves protein sample integrity by maintaining a higher sample buffer pH. The pH of the Novex® NuPAGE® sample buffer does not drop below pH 8 (even when heated to 70°C), so the protein sample does not degrade during sample preparation.

Novex® NuPAGE® gels also have a higher buffering capacity and are better at handling samples with different ionic strengths, compared to TGX™ gels. Novex® NuPAGE® gels are able to efficiently separate proteins in high ionic strength samples without band broadening or narrowing (Figures 1 and 2).

Furthermore, compared to gels that provide a similar separation profile, Novex® NuPAGE® gels contain larger effective pores (relative to Laemmli-type Tris-glycine gels with a similar separation profile), allowing proteins to be blotted more efficiently onto membranes (Figures 3 and 4).

The "fast run" protocol recommended for Novex® NuPAGE® gels delivers excellent performance, with straight bands and excellent resolution very similar to that achieved with the standard run conditions. When TGX™ gels are used with the fast run protocol recommended by Bio-Rad, they exhibit increased band curvature, band smearing, and distortion (Figure 2).

The Novex® NuPAGE® gels demonstrated consistent performance even after storage for 6 months, proving the stability conferred by the near-neutral pH of the Bis-Tris

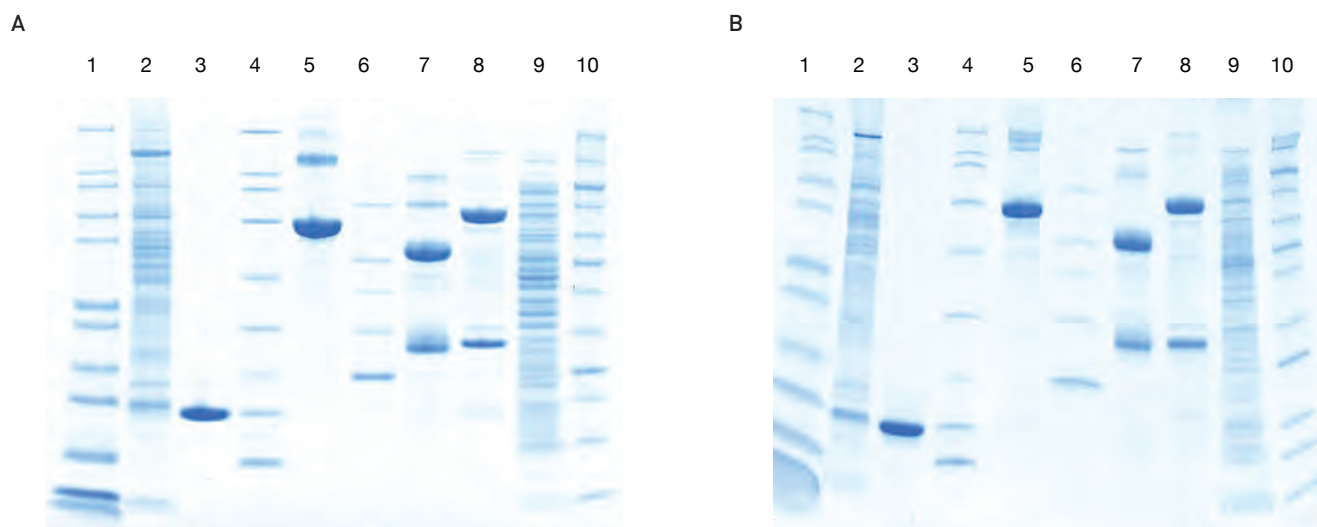


Figure 2. Performance comparison of Novex® NuPAGE® Bis-Tris gels and TGX™ gels under fast run conditions. (A) Novex® NuPAGE® gels resolved sharp protein bands under fast run conditions. (B) Bis-Tris gels exhibited band distortion and curvature under fast run conditions. Fast run protocols were executed according to each manufacturer's recommendations. Samples loaded: (1) Mark12™ Unstained Standard, 10 µL; (2) rat liver lysate, 10 µg; (3) lysozyme, 6 µg; (4) Bio-Rad® Broad Range Standard, 1:50 dilution; (5) BSA, 6 µg; (6) MagicMark™ XP Standard, 10 µL; (7) human IgG, 6 µg; (8) human IgM, 6 µg; (9) *E. coli* lysate, 10 µg; (10) Novex® Sharp Unstained Standard, 10 µL.

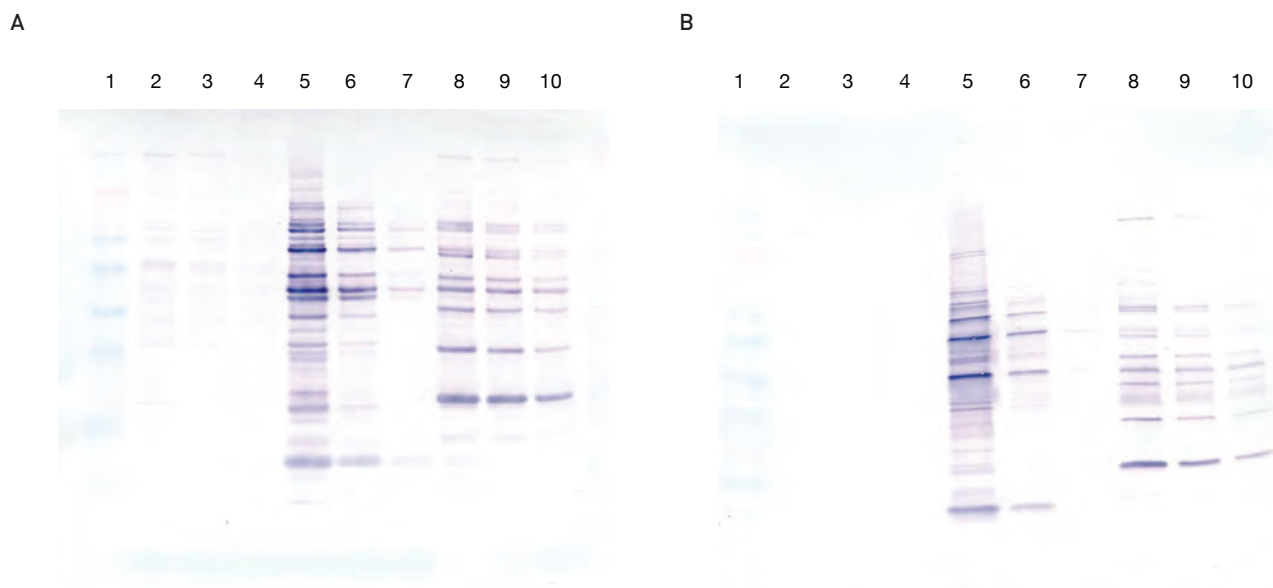


Figure 3. Novex® NuPAGE® 4–12% Bis-Tris gels and protocols (A) enable higher detection sensitivity than do 4–20% TGX™ gels and protocols (B). The following samples were loaded on each gel: (1) SeeBlue® Plus2 Pre-stained Standard, 3 μ L; (2) Bio-Rad® Broad Range Standard, 1:200 dilution; (3) Bio-Rad® Broad Range Standard, 1:400 dilution; (4) Bio-Rad® Broad Range Standard, 1:800 dilution; (5) *E. coli* lysate, 200 ng; (6) *E. coli* lysate, 20 ng; (7) *E. coli* lysate, 2 ng; (8) MagicMark™ XP Western Protein Standard, 1X (5 μ L); (9) MagicMark™ XP Western Protein Standard, 0.5X (2.5 μ L); (10) MagicMark™ XP Western Protein Standard, 0.25X (1.25 μ L). The membranes were detected using an anti-*E. coli* primary antibody and the WesternBreeze® Chromogenic Kit–Anti-Rabbit.

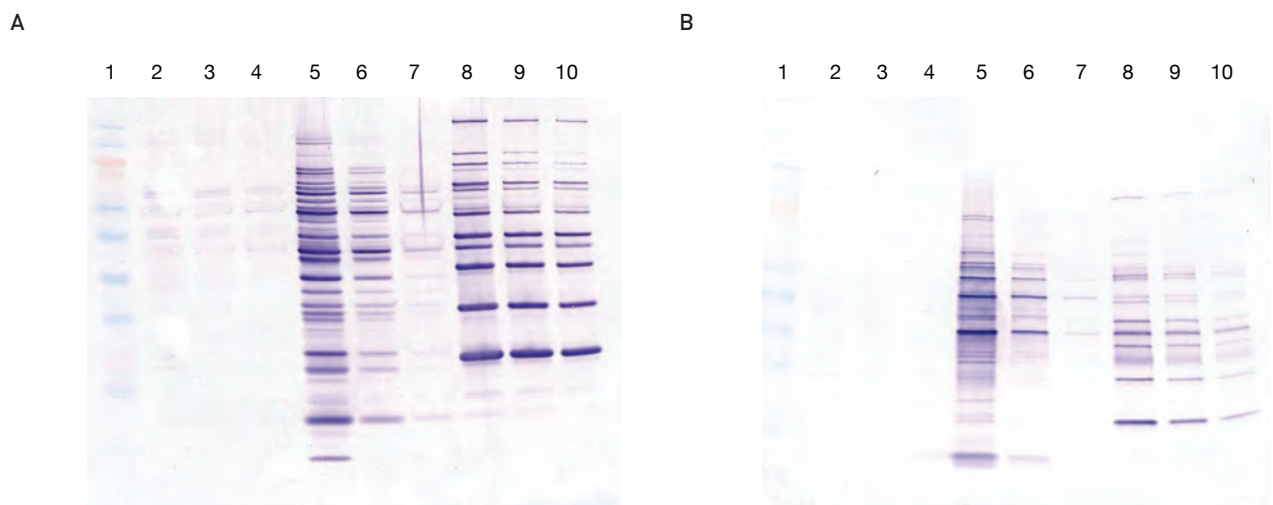


Figure 4. Novex® NuPAGE® 4–12% Bis-Tris gels (A) allow better transfer of proteins using the iBlot® Dry Blotting System than do 4–20% TGX™ gels (B). The following samples were loaded on each gel: (1) SeeBlue® Plus2 Pre-stained Standard, 3 μ L; (2) Bio-Rad® Broad Range Standard, 1:200 dilution; (3) Bio-Rad® Broad Range Standard, 1:400 dilution; (4) Bio-Rad® Broad Range Standard, 1:800 dilution; (5) *E. coli* lysate, 200 ng; (6) *E. coli* lysate, 20 ng; (7) *E. coli* lysate, 2 ng; (8) MagicMark™ XP Western Protein Standard, 1X (5 μ L); (9) MagicMark™ XP Western Protein Standard, 0.5X (2.5 μ L); (10) MagicMark™ XP Western Protein Standard, 0.25X (1.25 μ L). The membranes were detected using an anti-*E. coli* primary antibody and the WesternBreeze® Chromogenic Kit–Anti-Rabbit.

chemistry. In contrast, the performance of the TGX™ gels, notably the curving of protein bands, appears to suffer after 6 months of storage, which is less than half of Bio-Rad's stated shelf life (Figure 5).

These studies demonstrate that the Novex® NuPAGE® Bis-Tris gel system enables greater sample integrity, straighter bands, and higher western transfer efficiency compared to the Bio-Rad® TGX™ gel system.

Materials and methods

Standard protocols

Protein samples were prepared according to each manufacturer's recommended protocols. Samples separated on the TGX™ system (TGX™ samples) were prepared in Laemmli Sample Buffer. DTT was added to a final concentration of 175 mM, and the samples were reduced for 5 minutes at 95°C. Samples separated on the Novex® NuPAGE® system were prepared in Novex® NuPAGE® LDS Sample Buffer (Cat. No. NP0008) with 50 mM DTT and heated at 70°C for 10 minutes. The TGX™ samples were loaded on a 4–20% TGX™ gel and run with Tris/glycine/SDS buffer (Bio-Rad). The Novex® NuPAGE® samples were loaded on

a Novex® NuPAGE® 4–12% Bis-Tris gel (Cat. No. NP0321BOX) and run with 1X MES SDS buffer (Cat. No. NP0002). In the standard process, both gels were run at 200 V (as recommended by each manufacturer) until the dye front reached the bottom of the gel. The gels were washed 3 times for 5 minutes each in water, stained with SimplyBlue™ SafeStain (Cat. No. LC6060) for 1 hour, and then destained overnight in water. A second 1-hour wash in water was performed before imaging.

Fast run protocols

The 4–20% TGX™ gels were run with 1X Tris/glycine/SDS buffer (Bio-Rad) at 300 V (constant voltage), and the separation was completed in 18 minutes. The Novex® NuPAGE® 4–12% Bis-Tris gels were run at 250 V (constant voltage), and the separation was completed in 24 minutes. Each supplier's gel boxes and buffers were used for their respective gels.

Wet transfer

The Novex® NuPAGE® 4–12% Bis-Tris gel was transferred using 1X Novex® NuPAGE® Transfer Buffer (Cat. No. NP0006) with 10% methanol and a 1:1,000 dilution of Novex® NuPAGE® Antioxidant (Cat. No. NP0005) for 1

hour at 30 V. The 4–20% TGX™ gel was transferred using Towbin transfer buffer with 20% methanol and run at 30 V for 1 hour. All wet transfers were performed using the Xcell II™ blot module.

iBlot® dry transfer

Both gels were transferred under the same conditions using iBlot® Transfer Stacks, Mini Nitrocellulose (Cat. No. IB3010-02), on the iBlot® Dry Blotting System. The standard P3 program (20 V) was applied to both gel types for 7 minutes.

Immunodetection

After transfer to a nitrocellulose membrane, the proteins were detected using the WesternBreeze® Chromogenic Kit–Anti-Rabbit (Cat. No. WB7105). Rabbit anti-*E. coli* antibody (Cat. No. B0357, Dako) diluted to 1:1,000 was used for the detection. All reagents needed for the detection were prepared as specified in the WesternBreeze® manual. The blot processing was executed using the BenchPro® 4100 Western Processing System and the standard WesternBreeze® protocol preprogrammed into the instrument. All membranes were incubated for 40 minutes with the detection substrate and then rinsed with water to stop the reaction.

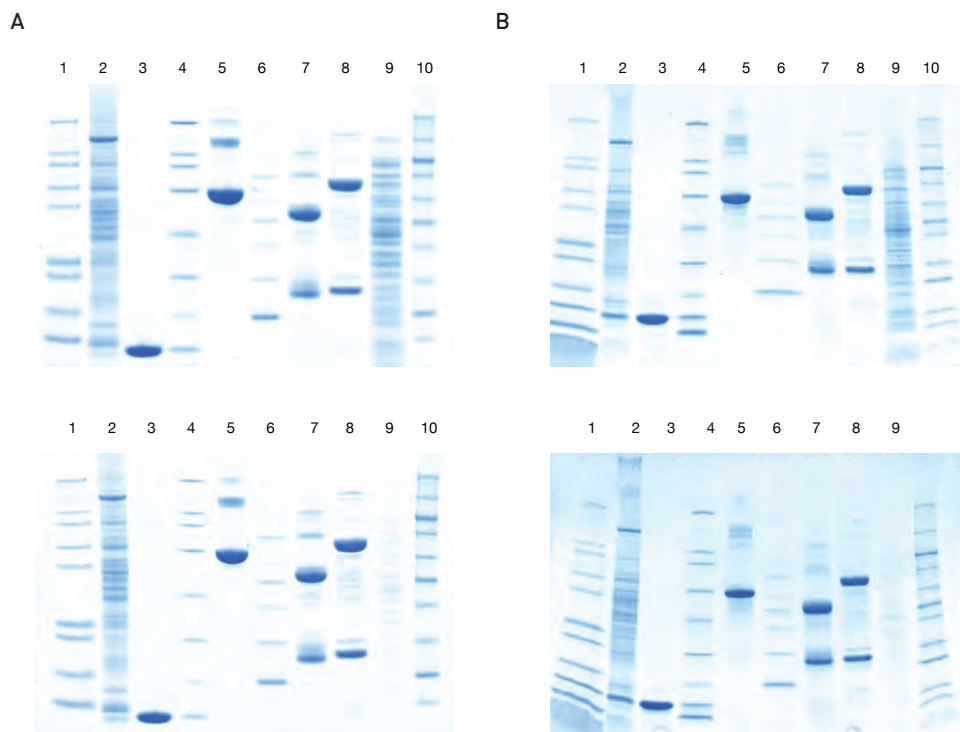


Figure 5. Novex® NuPAGE® Bis-Tris gels (A) demonstrate superior stability after prolonged storage, compared to Bio-Rad® TGX™ gels (B). Novex® NuPAGE® and TGX™ gels were run within 1 month of receipt (top) and after 6 months of storage (bottom) according to the manufacturer's recommendations. Samples loaded: (1) Mark12™ Unstained Standard, 10 μ L; (2) rat liver lysate, 10 μ g; (3) lysozyme, 6 μ g; (4) Bio-Rad® Broad Range Standard, 1:50 dilution; (5) BSA, 6 μ g; (6) MagicMark™ XP Standard, 10 μ L; (7) human IgG, 6 μ g; (8) human IgM, 6 μ g; (9) *E. coli* lysate, 10 μ g; (10) Novex® Sharp Unstained Standard, 10 μ L.

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