ELECTROPHORESIS

Supporting Information for Electrophoresis

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Edita Aksamitiene, Jan B. Hoek, Boris Kholodenko and Anatoly Kiyatkin

Multistrip Western blotting to increase quantitative data output

SUPPORTING INFORMATION

INHOMOGENEOUS GEL AND SEPARATE TRANSFER CONDITIONS CAUSE SIGNIFICANT SIGNAL VARIABILITY

The size of midi-gels dramatically augments errors caused by increased gel heterogeneity combined with unequal electrophoresis and protein transfer conditions (Fig. 1S). Non-uniform heat distribution through the long gel matrix and slightly slower migration of first and last lanes during electrophoresis due to inhomogeneous electric field caused dissimilar signal intensities from identical samples that were randomly loaded in different lanes. The optimal protein transfer region covers approximately ten neighboring lanes in the center and is less adequate on the edges of the midi-gel.

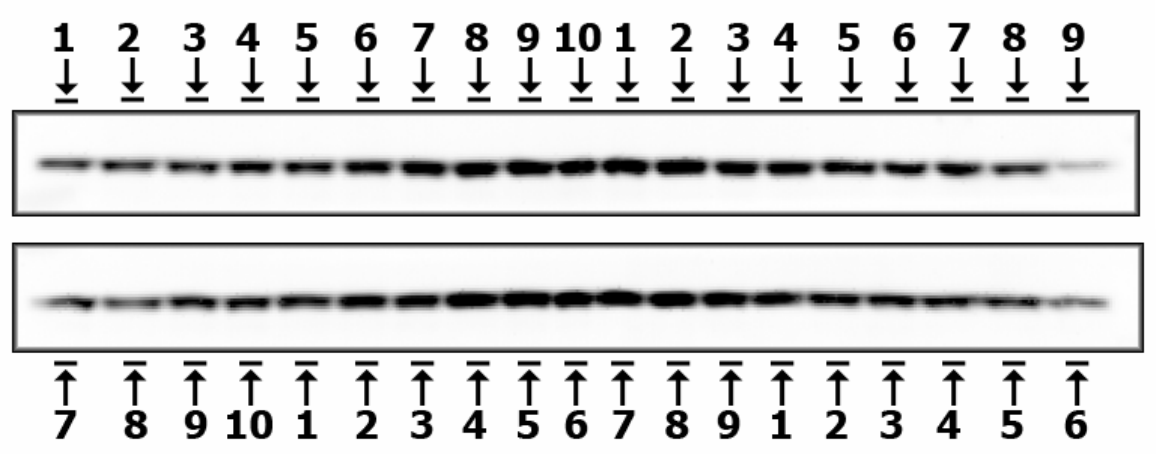

Fig. 1S. A549 cells were cultured and lysed as described under section "Materials and Methods". Ten unique samples were prepared (indicated as digits). Equal volume of the same lysate was loaded in two non-neighboring wells of Novex 4-12% gradient Bis-Tris 20-well midi-gels. Proteins were resolved by LDS-PAGE under reducing conditions and independently transferred onto nitrocellulose membranes in Criterion Cell Blotter (30V constant for 1.5 hours). The blocked membranes were blotted with polyclonal antibodies that recognize 28 kDa GRB2 at a dilution 1:2,000 with successive procedures described in "Materials and Methods" and collectively exposed. A representative experiment is shown, but similar results were observed in seven separate experiments, including three performed with Novex 14% Tris-Glycine 15-well gels.

GEL-RELATED BAND ARTIFACTS THAT IMPAIR QUALITY OF THE SIGNAL

A wide variety of precast gels is commercially available that vary in gel composition, percentage, thickness, number of well and buffer systems. The major disadvantage of commercially obtained gels is variability in batch-to-batch quality. Despite these drawbacks, precast gels save time and have lower gel-to-gel variations than self-prepared gels. The proteins resolved by high quality gels in freshly prepared buffer should result in crisp and straight bands that are not distorted (Fig. 2SA-B). The older a gel, the higher the number of poor quality bands was detected in our experiments. Old Bis-Tris or Tris-Glycine midi-gels gave mostly winged and wavy bands; old Tris-Glycine mini-gels usually provided faded, fuzzy and ribbon-like bands, while faint, somewhat faded, winged or smiley bands were associated with old Bis-Tris mini-gels (Fig. 2SA). In general, 4-12% gradient NuPAGE mini-gels, which are fairly homogenous, have long shelf life, demonstrated excellent protein separation and were suitable to generate better quality bands in comparison with regular Tris-Glycine gels even after their expiration date (Fig. 2SB).

There are many other factors that influence band quality. For example, an excess of SDS in transfer buffer and lack of contact between gel and the membrane during transfer may result in bubbly and dotted bands. Wavy, faint or diffuse bands may occur, when insufficient protein is loaded, protein binding to the membrane is weak, there is a variation in pressure between the gel and the membrane during transfer, or the time of transfer for certain molecular weight protein is not optimized. The use of fresh and high quality SDS in sample preparation markedly increases the sharpness of bands. Ribbon-like, dotted, sliced and streaked bands may form as a result of poor transfer conditions but typically require checking concentration and potency of primary antibodies. Streaked bands may also occur due to poor separating gel quality, leading to non-uniform protein concentration across the lane or high sample viscosity. Non-uniform heat distribution through the gel matrix and slightly slower

migration of first and last lanes during electrophoresis due to inhomogeneous electric field may cause smiley bands, occurring at the base and at the edges of the gel. Overheated gel looses rigidity, leading to poor resolving and blurry bands. Incorrect sample buffer-to-protein ratio, overheating during preparation step, failure to remove insoluble material, overloading or underloading protein sample may also cause band artifacts.

Although the visualization of most bands is adequate to confirm “yes-or-no” protein expression or compare “on-off” cell responses to a stimulus, only intense bands with well-delineated borders should be used for quantitative analysis.

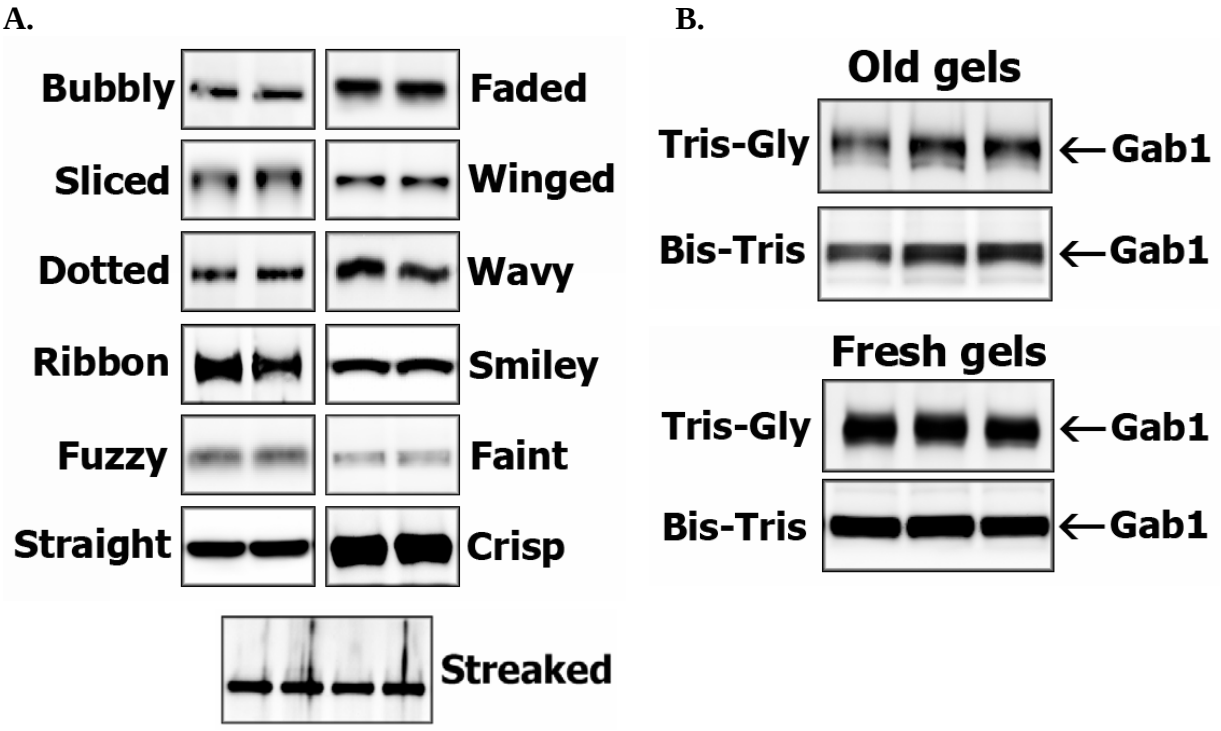

Fig. 2S. A. Observed artifacts of protein bands in Western blotting. **B. Comparison of band quality in old and fresh gels**. 105 kDa GAB1 protein was detected in HEK293 cell lysates, resolved by 8% Tris-Glycine and 4-12% gradient Bis-Tris 10-well gels used two months before and after their expiration date.

INTERFERENCE OF NON-SPECIFIC BANDS MAY BE BYPASSED BY NARROWING DETECTION AREA

The large surface of gel-sized membranes is often unequally covered with immunoblotting reagents, increasing uneven background. When whole membrane is immunoblotted for a single protein, poor antibodies may additionally cause the occurrence of non-specific bands that interfere or overshadow weaker specific signals (Fig. 3S). Cutting a gel strip with a protein of interest in the middle helps to concentrate antibodies on the more narrow area of the membrane in subsequent incubation steps after protein transfer, and to diminish these drawbacks.

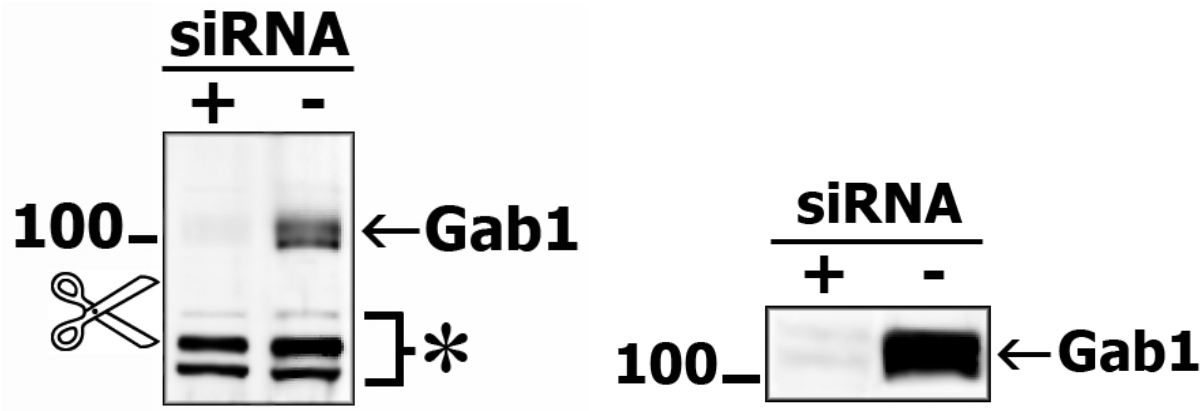

Fig. 3S. HEK293 cells were transfected with GAB1-specific small interfering RNA (siRNA) for 72 hours as described previously [16]. The cell lysates were prepared, separated by SDS-PAGE and immunoblotted for total GAB1 protein as described in “Materials and Methods”. Arrow sign shows 105 kDa GAB1 protein, which was specifically suppressed by siRNA. Scissors in the left panel indicates the suggested cutting area of the gel in order to eliminate the detection of non-specific bands (indicated by asterisk symbol). The improved signal after elimination of non-specific bands is shown in the right panel.