Research

Phos-tag™ SuperSep Precast Gels FAQ



Q1. What electrophoresis tanks are the Phos-tag Precast Gels compatible with?

Phos-tag Precast Gels are available in three cassette sizes:
[83 x 100 x 3.9 mm] is suitable for Bio-Rad Mini-PROTEAN Tank.
[100 x 100 x 3.0 mm] is suitable for Wako Easy Separator.
[100 x 100 x 6.6 mm] is suitable for Invitrogen® XCell SureLock® Mini-Cell Tank.

Q2. What gel staining methods can we use?

CBB, negative, silver, and fluorescent staining methods can be used.

Q3. CBB-stained gel cannot be de-stained clearly.

A microwave can be used when de-staining to give you a clearer gel.

Procedure: Transfer the stained gel in 100 mL of deionized water in the container, add a few rolls of Kim-Wipes, and heat it in a microwave for a few minutes. Replace deionized water and repeat the same procedure 2-3 times. Be careful with handling as the container will be hot.

Q4. Can the product be used for Western blot?

Yes: However, zinc in the gel must be removed by EDTA treatment to achieve optimal transfer efficiency to the membrane.

Procedure: Immerse the gel in a transfer buffer (25 mmol/L Tris, 192 mmol/L Glycine, 10% MeOH) containing 10 mmol/L EDTA and agitate gently for 10 minutes. Repeat the procedure 2 times. Replace with a transfer buffer (25 mmol/L Tris, 192 mmol/L Glycine, 10% MeOH) that does not contain EDTA, agitate for 10 minutes, and transfer onto PVDF membrane or nitrocellulose membrane. If the transfer efficiency is poor, consider increasing the frequency of EDTA treatment or the concentration of EDTA.

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Q5. Bands are distorted.

Distortion and tailing of bands can be observed with samples that contain EDTA, inorganic salts, and detergents. In such cases, it is effective to desalt samples by dialysis or precipitation with TCA. The presence of an empty lane can also cause band distortion. Apply a sample buffer (1x) in empty lanes to avoid distortion.

Q6. Phosphorylated and dephosphorylated target proteins are not separated.

Perform electrophoresis and confirm band shift with ß-casein (positive control) and alkaline phosphatase-treated ß-casein (negative control). If there is a band shift, it is likely that phosphorylated and dephosphorylated variants of your target protein are not separated with the Phos-tag and/or acrylamide concentrations of this product.

Q7. Can we use crude extracts of cells with this product?

Yes: However, the use of proteins with small Rf values may result in unclear bands.

Q8. What is the appropriate amount of a sample that should be applied to a well?

1-5 μg for purified proteins with CBB staining, and 10-30 μg for tissue or cell extracts (adjust the amount depending on the level of protein expression). These are only recommended amounts. SDS-PAGE or Western blot should be performed to determine the appropriate amount of a sample.

Q9. What molecular markers should we use?

There are no specific markers that we recommend as the product is not influenced by the molecular weight of a marker. Instead of a marker, E. coli -derived recombination proteins or dephosphorylated samples are recommended as negative controls.

Q10. Which ion complex is contained in the product? The product contains zinc ion.

Q11. How can we determine whether the band shift is caused by phosphorylation?

Perform gel electrophoresis with 12.5% SuperSep™ Ace (Cat. No. 199-14971) with the same gel concentration to confirm that the target protein is not degraded.

Q12. Do we need to cool the system down (by running the gel in a cold room or using an ice box)?

The SDS-PAGE can be performed at room temperature.

Q13. Our sample buffer for loading protein samples on the Phos-tag gel contains 2-mercapthoethanol. Is it possible to use DTT instead?

Using DTT instead of 2-ME does not cause any issues. For reference, the following paper describes a self-prepared 2X sample buffer solution containing DTT, which was used in combination with the Phos-tag gel (195-17991) and showed reliable results: Monitoring cellular energy balance in single cells using fluorescent biosensors for AMPK - PMC . The composition of the 2X sample buffer solution in this paper is as follows:130 mM Tris (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol, 200 mM DTT, 0.1% (w/v) Bromophenol Blue (BPB)

Q14. Our protein samples contain 0.05-0.1% of Tween-20 and 0.5M NaCl. Is it compatible with loading on Phos-tag gels or the Phos-tag agarose column?

The effect of NaCl is not confirmed, but 0.05-0.1% of Tween-20 is acceptable.

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