



## Nucleic Acid Stain Troubleshooting Guide For most common agarose gel staining issues

### NOTE:

- ✓ In case of any problems appearing with the gel quality, please read the product manual carefully again to refresh the recommended protocols and important product usage tips.
- ✓ Compare the recommended protocols with the ones you use and draw appropriate conclusions. Repeat the experiments with fresh buffers/gels by following protocols strictly as recommended.

### The most important product properties and protocols to follow:

#### Storage:

- ✓ Store the stain strictly at +15–25 °C in the dark.
- ✓ The stain is sensitive to light and should be protected from light all the time during storage, usage and gel staining. Keep the trays with the staining buffer protected from light f.e. covered during the staining and between stainings.
- ✓ The stain is stored at ambient temperature at about +20°C. Due to common cooled shipments, it can be exposed for a short time to +4°C. If accidentally shipped with cooling packs at +4°C, warm the stain solution shortly to maximal 30°C and mix well to dissolve all precipitate. And keep it further only at ambient temperature.
- ✓ Never freeze the product. Discard if frozen. If once frozen, the new product shall be ordered.

#### Usage:

- Use 8-10 µl of the stain solution per 100 ml of the gel right before casting the gel.
- Add stain to the gel solution only after agarose cools to hand-warm temperature (~40–50 °C) and mix well.
- Optionally, if low NA concentration is expected, add 2-5 µl of the stain solution per 100 ml of the 1X electrophoresis running buffer.
- Gel destaining is not needed, but it might help to reduce the background. For optional destaining, keep the stained gel in fresh water or 1X electrophoresis buffer batch while slowly shaking for 10 minutes.
- If you reuse the molten gels, add at least half a portion of the stain each time after boiling and cooling the gel solution down. Reused gels might have higher background staining.
- For post-run staining, soak the gel for 10-30 minutes into the 100 ml solution of 1X electrophoresis buffer freshly mixed with 10 -30 µl of the stain. The amount of the stain can be adjusted, as the staining intensity depends on gel percentage and thickness. Thick and high percentage gels require more stain and longer staining time.
- Keep the tray protected from light (f.e., covered) during the staining and between stainings.
- The same staining solution can be used for up to 5-10 gels, or up to the sensitivity is acceptable.
- Longer staining time of 30 minutes gives better results when detecting low DNA amounts, however, it may cause background or diffusion of smallest NA fragments.
- To avoid stain absorbance on plastic or glass during the storage, make a fresh staining solution every day.

## Short Nucleic Acid Stain Troubleshooting:

| Problem   | Likely Cause   | Solution  |
|---|--|---|
| <b>DNA bands too faint, low staining sensitivity</b>          | <ol style="list-style-type: none"> <li>1. Stain degraded due to cooling/freezing or light exposure</li> <li>2. Stain not mixed well before adding</li> <li>3. Stain added to too hot agarose and inactivated</li> <li>4. Insufficient stain amount or too short post-run gel staining time</li> <li>5. In case of post-run staining, stain absorbed by a bad quality plastic of the staining tray</li> <li>6. Weak or uneven stain distribution in the agarose or the stain was partially precipitated</li> <li>7. No dye added to running buffer when DNA concentration is low</li> </ol> | <ol style="list-style-type: none"> <li>1. Store at +15–25 °C in the dark; discard if frozen. Keep trays with staining buffer protected from light during the staining and between stainings</li> <li>2. Mix gently and thoroughly before every pipetting</li> <li>3. Add stain only after agarose cools to hand-warm (~40–50 °C)</li> <li>4. Increase stain volume slightly (if you used minimal amount) or extend post-run staining for a few min</li> <li>5. If possible, use high quality non-binding plastic trays for staining the gels. If the staining buffers shall be reused several times, protect from light</li> <li>6. Warm stain shortly to 30°C if precipitate is visible and mix until fully homogeneous. Mix the stain well in agarose solution or staining buffer by slight shaking.</li> <li>7. Add the stain not only into the gel solution, but also use 2–5 µl per 100 ml electrophoresis running buffer for low-abundance samples</li> </ol> |
| <b>Smearred or distorted bands</b>                            | <ol style="list-style-type: none"> <li>1. Stain added to very hot agarose (damaged)</li> <li>2. Uneven stain distribution in precast gel</li> <li>3. DNA overloaded or contaminated with proteins or nucleases</li> <li>4. Electrophoresis voltage too high</li> <li>5. Gel-wells were not formed well or were leaky</li> </ol>  | <ol style="list-style-type: none"> <li>1. Add stain only after agarose cools to hand-warm (~40–50 °C)</li> <li>2. Mix gel solution gently and avoid bubbles</li> <li>3. Reduce DNA amount loaded, increase the sample volume by adding sample loading buffer. If nucleases are suspected, change the sample loading buffer, and make new gel and new electrophoresis buffer.</li> <li>4. Run the gel at ~110–130 V</li> <li>5. Cast a new gel carefully and repeat the experiment</li> <li>6. If problems persist, try rather post-staining instead of in-gel staining</li> </ol>   |
| <b>Excessively high gel background</b>                        | <ol style="list-style-type: none"> <li>1. Too much stain in gel or buffer</li> <li>2. Stain exposed to cold or light</li> <li>3. Thick gel or long staining time increasing background fluorescence</li> </ol>   | <ol style="list-style-type: none"> <li>1. Reduce stain amount by 20–50% for thin and low percentage gels</li> <li>2. Keep gel and stain solutions protected from light at room temperature</li> <li>3. Shorten post-run staining time to 10–20 min. Use optional destaining (soak in water or fresh electrophoresis buffer for 5–15 min) to reduce background</li> </ol>  |
| <b>Precipitation in stain stock</b>                           | <ol style="list-style-type: none"> <li>1. Temporary exposure to cold (e.g., 4 °C) for example if the stain is used/stored in cold laboratory rooms</li> </ol>  | <ol style="list-style-type: none"> <li>1. Warm to +25–30 °C until dissolved; mix gently. If completely frozen at any point - discard the stain and order the new one.</li> </ol>  |
| <b>Weak fluorescence with reused agarose or reused buffer</b> | <ol style="list-style-type: none"> <li>1. Not enough stain added after reheating the gel in case of gel staining</li> <li>2. Stain degraded by repeated heating cycles in case of reusing the agarose</li> <li>3. Stain used-up in case of reusing the same buffer with the post-run protocol for multiple gels stainings</li> </ol>   | <ol style="list-style-type: none"> <li>1. Preferably, always use freshly made gel with freshly added stain or fresh buffer for post-run staining. If reuse is wanted - add at least half the original stain dose after each re-melt of agarose.</li> <li>2. Add stain only after agarose cools to hand-warm (~40–50 °C). But note- remelting and adding the stain will increase the background staining.</li> <li>3. You can use the same staining buffer for several gels until the staining efficiency is enough. As the stain is binding to the DNA and gel, after several uses, add a little bit of stain again and use further. But note - the buffer shall be kept away from light exposure and only high-quality plastic or glass trays shall be used to avoid stain absorption.</li> </ol>  |