Detection Buffer Formulations

General Detection Buffer

**Tris-buffered saline (TBS), 2 L**
20 mM Tris-HCl, 500 mM NaCl (pH 7.5)
(catalog #170-6435, 1 L, 10 x)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>4.84 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>58.48 g</td>
</tr>
<tr>
<td>diH₂O</td>
<td>1.5 L</td>
</tr>
</tbody>
</table>

Adjust pH to 7.5 with HCl.
Adjust volume to 2 L with diH₂O.

**TTBS wash solution, 1 L**
20 mM Tris-HCl, 500 mM NaCl, 0.05% Tween 20 (pH 7.5)
0.5 ml Tween 20
1 L TBS

**Citrate-buffered saline (CBS)**
20 mM citrate, 500 mM NaCl (pH 5.5)
Included in Immun-Blot® protein G kits.

**TCBS wash solution, 1 L**
20 mM citrate, 500 mM NaCl, 0.05% Tween 20 (pH 5.5)
0.5 ml Tween 20
1 L CBS

**Blocking solution, 100 ml**
3% gelatin-TBS
Add 3.0 g gelatin to 100 ml TBS.
Heat to 50°C; stir to dissolve.

or

3% BSA-TBS
Add 1.0 g BSA to 100 ml TBS; stir to dissolve.

or

5% nonfat milk-TBS
Add 0.5 g nonfat dry milk to 100 ml TBS; stir to dissolve.

**Antibody dilution buffer, 200 ml**

1% gelatin-TTBS
Add 2.0 g gelatin to 200 ml TTBS.
Heat to 80°C; stir to dissolve.

or

3% BSA-TTBS
Add 6.0 g BSA to 200 ml TTBS; stir to dissolve.

or

5% nonfat milk-TTBS
Add 10.0 g nonfat dry milk to 200 ml TTBS; stir to dissolve.

**Antibody buffer (for chemiluminescence, ImmunStar™ AP only)**
0.2% nonfat milk-TTBS
Add 0.4 g nonfat milk to 200 ml TTBS; stir to dissolve.

**Antibody buffer for protein G-HRP, 100 ml**

1% gelatin-TCBS
Add 1.0 g gelatin to 100 ml TCBS.
Heat to 50°C; stir to dissolve.

**Protein G-HRP conjugate solution, 100 ml**
Mix 33 µl protein G conjugate solution in 100 ml 1% gelatin in TCBS.

**Streptavidin-biotinylated AP complex, 100 ml**
33 µl streptavidin
100 ml TTBS
33 µl biotinylated AP
Incubate the complex 1–3 hr at room temperature before use.

---

Note: Gelatin can clog membranes and cut off the vacuum flow of microfiltration units; use an alternative blocking solution with the Bio-Dot or Bio-Dot SF apparatus.

Note: Nonfat milk is not recommended for avidin/biotin systems as milk contains endogenous biotin and may cross-react with avidin-containing components in the detection system.
### Total Protein Staining Buffers and Solutions

#### Amido black staining solution, 1 L

**For nitrocellulose:**
- Amido black: 5 g
- Methanol: 400 ml

Adjust volume to 1 L with diH₂O.

or

- Amido black: 5 g
- Isopropanol: 250 ml
- Acetic acid: 100 ml

Adjust volume to 1 L with diH₂O.

**For PVDF:**
- Amido black: 1 g
- Methanol: 400 ml
- Acetic acid: 100 ml

Adjust volume to 1 L with diH₂O.

#### Amido black destain solution, 1 L

**For nitrocellulose:**
- Isopropanol: 250 ml
- Acetic acid: 100 ml

Adjust volume to 1 L with diH₂O.

**For PVDF:**
- Methanol: 400 ml
- Acetic acid: 100 ml

Adjust volume to 1 L with diH₂O.

#### Coomassie Blue R-250 staining solution, 1 L

**Coomassie Blue R-250:** 1 g
- Methanol: 400 ml
- Acetic acid: 100 ml

Adjust volume to 1 L with diH₂O.

#### Coomassie Blue R-250 destaining solution, 1 L

- Methanol: 400 ml
- Acetic acid: 100 ml

Adjust volume to 1 L with diH₂O.

#### Ponceau S staining solution

- Ponceau S: 2 g
- Trichloracetic acid (TCA): 30 g
- Sulfoalicylic acid: 30 g
- diH₂O: 80 ml

#### Ponceau S destaining solution

1% acetic acid or PBS

#### SYPRO Ruby blot pretreatment solution

- Acetic acid: 70 ml
- Methanol: 100 ml
- diH₂O: 830 ml

#### Colloidal gold blot staining solution

Use TTBS wash solution.

---

### Substrate Buffers and Solutions

#### HRP Substrate Buffers

- **4-(chloro-1-naphthol):**
  - 4CN: 60 mg
  - Methanol: 20 ml
  - Protect mixture from light.
  - 3% H₂O₂: 600 μl
  - Substrate solution: 100 ml

Mix the two solutions together.

Use immediately. Alternatively, use HRP conjugate substrate solution in kit format.

- **HRP conjugate solution:**
  - Dissolve contents of premixed substrate color development buffer in diH₂O to 1 L
  - Color reagent B: 600 μl
  - Development buffer: 100 ml
  - HRP color reagent A: 20 ml

Use immediately.

#### Diaminobenzidine (DAB)

- DAB: 50 mg
- TBS: 100 ml
- 3% H₂O₂: 100 μl

Use immediately.

#### AP Substrate Buffers

- **AP color development buffer:**
  - MgCl₂: 0.233 g
  - Tris base: 12.1 g
  - diH₂O: 800 ml

Adjust pH to 9.5 with HCl; adjust volume to 1 L with diH₂O.

- **5-bromo-4-chloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT):**
  - Dimethylformamide: 0.7 ml
  - phosphate/nitroblue tetrazolium: diH₂O: 0.3 ml
  - BCIP: 30 mg
  - Dimethylformamide: 1 ml
  - NBT: 15 mg

Add both solutions to 100 ml AP color development buffer. Use immediately. Alternatively, use AP conjugate substrate solution in kit format.

- **Immun-Star AP substrate solution (kit format):**
  - Use 5 ml chemiluminescent substrate per 100 cm².

For nitrocellulose membrane blots: Add 500 μl enhancer reagent to 10 ml Immun-Star chemiluminescent substrate. Store at 4°C for up to 1 week.

For PVDF membrane blots: Immun-Star AP generates a very fast light signal on PVDF membrane; therefore, the use of an enhancer is not necessary. The substrate is provided ready to use.

- **Immun-Star HRP substrate solution (kit format):**
  - A 1:1 mixture of luminol/ enhancer to peroxide buffer is recommended.
  - Use 10 ml per 100 cm² of membrane (12 ml for one 8.5 × 13.5 cm Criterion™ blot).

#### Stripping Buffer

- **Acidic glycine stripping buffer:**
  - Glycine: 7.5 g
  - Mg(CH₃COO)₂·4H₂O: 4.3 g
  - KCl: 3.7 g
  - diH₂O: 800 ml

Adjust pH to 2.2 with HCl. diH₂O to 1 L
This is an excerpt from Bio-Rad’s comprehensive Protein Blotting Guide (Bulletin 2895).