

IHC: Antigen Retrieval Protocols

Some antibodies require an additional antigen retrieval (AGR) step for efficient antigen detection.

These protocols can be used for formaldehyde fixed tissue sections that are not paraffin embedded. Please refer to the **remarks** section for immunohistochemistry (IHC) on the corresponding data sheet to choose the correct AGR protocol.

Antigen retrieval with citrate buffer pH 6.0

Materials and reagents

- **AGR buffer:** 10 mM citrate, pH 6.0 without detergent
- **TBS:** Tris buffered saline, 50 mM Tris pH 7.2/150 mM NaCl

Procedure

1. Incubate free floating sections or sections on slides overnight at 60°C in **AGR buffer**.
2. Let cool down to room temperature.
3. Wash three times with **TBS** for 10 min each.
4. Continue with the blocking step of the staining procedure.

Antigen retrieval with Tris-EDTA buffer pH 9.0

Materials and reagents

- **AGR buffer:** 10 mM Tris, 1 mM EDTA, pH 9.0 without detergent
- **TBS:** Tris buffered saline, 50 mM Tris pH 7.2/150 mM NaCl

Procedure

1. It is important to work with sections on slides to preserve tissue integrity as much as possible.
2. Incubate sections overnight at 60°C in **AGR buffer**.
3. Let cool down to room temperature.
4. Wash three times with **TBS** for 10 min each.
5. Continue with the blocking step of the staining procedure.

Antigen retrieval with pepsin

Materials and reagents

- **AGR solution:** 0.2 mg/ml pepsin (histology grade) in 0.2 M HCl
- **TBS:** Tris buffered saline, 50 mM Tris pH 7.2/150 mM NaCl

Procedure

1. Incubate free floating sections or sections on slides for 20 min at 37°C in **AGR solution**.
2. Wash three times with **TBS** for 10 min each.
3. Continue with the blocking step of the staining procedure.

Antigen retrieval with methanol/acetic acid

Materials and reagents

- **AGR solution:** 95% methanol, 5% acetic acid stored at -20°C
- **TBS:** Tris buffered saline, 50 mM Tris pH 7.2/150 mM NaCl

Procedure

1. Incubate free floating sections or sections on slides for 10 min at -20°C in **AGR solution**.
2. Wash three times with **TBS** for 10 min each.
3. Continue with the blocking step of the staining procedure.

Antigen retrieval with formic acid

Materials and reagents

- **AGR solution:** formic acid
- **TBS:** Tris buffered saline, 50 mM Tris pH 7.2/150 mM NaCl

Procedure

1. Incubate free floating sections or sections on slides for 3 min at room temperature in **AGR solution**.
2. Wash three times with **TBS** for 10 min each.
3. Continue with the blocking step of the staining procedure.