**SILVER STAINING COMPATIBLE WITH MS ANALYSIS**

**FIXING of the gel**

Remove and immerse the gel in a fixing solution (FIX):

**FIX**: 50 % methanol (MetOH)

10% acetic acid

Leave the gel in FIX for 10 min, remove the FIX solution, wash to neutral pH and proceed with staining.

It is possible to color the gel after the Coomassie staining (if this is too little sensitive), in this case it is necessary to wash in water 3 times for 10 min until it has a neutral pH (6-7) and it is not necessary to repeat the fixing.

**SILVER STAINING**

Before proceeding with staining, make sure that the aqueous solution in which the gel is immersed is at neutral pH, otherwise wash again.
Solutions to prepare (prepare AgNO3 and development solutions freshly):

 **Sensit**: Na2S2O3 0,02% (0,1g in 500ml) 🡪 it can be kept at 4 °C and used cold

**Silver**: AgNO3 0,2% (0,1g in 50 ml)

**Develop**: Vtot=50 ml 1,5g Na2CO3 + 1 ml Na2S2O3 0,02% 100l formaldeide 37% (never use glutaraldehyde: it is not compatible with MS!)

**Blocking:** Acetic acid 6%

Sequence:

* immerse the gel in the **Sensit** sol (Na2S2O3) for 2-3 min while stirring;
* do 2-3 quick washings in H2O (2-3 min each);
* immerse the gel in the Silver solution (AgNO3) for 25 min while stirring;
* do 2-3 quick washings in H2O (2-3 min each);
* immerse the gel in the **Development sol**, stirring a little until optimal contrast: if the solution turns yellow, renew the Development sol in which the gel is immersed.
* when the contrast is considered optimal, quickly remove the Development sol and replace it with the **Blocking sol**. The gel can be stored in this solution or in water (in this last case, in particular, storage at 4 ° C is suggested to prevent the formation of mold).

N.B .: Carefully follow the **Guidelines** to avoid contamination.