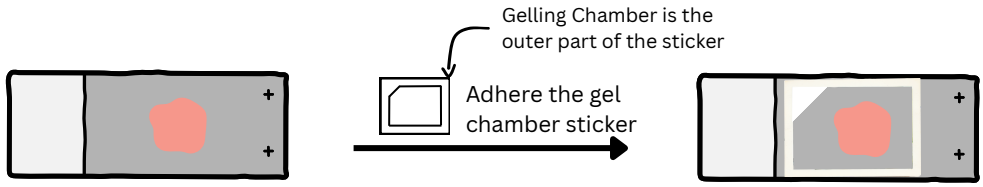
Magnify™ Kit User Guide

FFPE tissues, v1.26.05

Video Demo

Step 1. Follow standard protocol (e.g., https://www.abcam.com/protocols/ihc-deparaffinization-protocol) to de-paraffinize the FFPE tissue slide. Use a Kimwipe to remove any residual liquid surrounding the tissue. Choose the smallest gel chamber sticker that accommodates the tissue size and adhere it to the dried surface around the tissue.

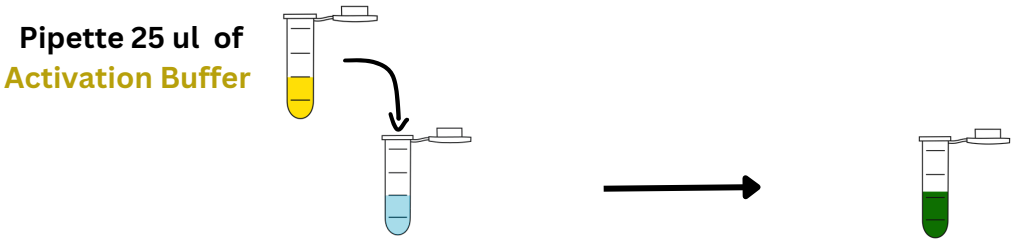


De-paraffinized FFPE tissue

Step 2. Take out Solution A and Solution G from the fridge. Pipette 500 uL of Solution G into an empty 2 mL Eppendorf tube (not provided).

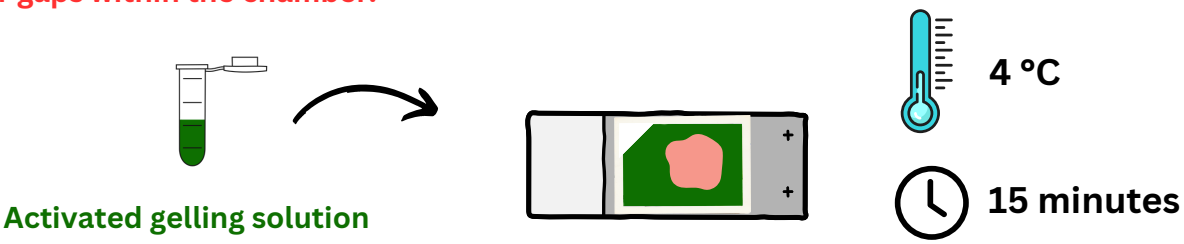
Step 3. Pipette 600 ul of Solution A into a provided Activation tube and vortex to make the **Activation Buffer.**

Step 4. Pipette 25 uL of the Activation Buffer to the 500 uL of Solution G to make **Activated Gelling Solution.** **Caution: The Activated Gelling Solution is not stable and it should be used within 15 mins before premature gelling happens.**



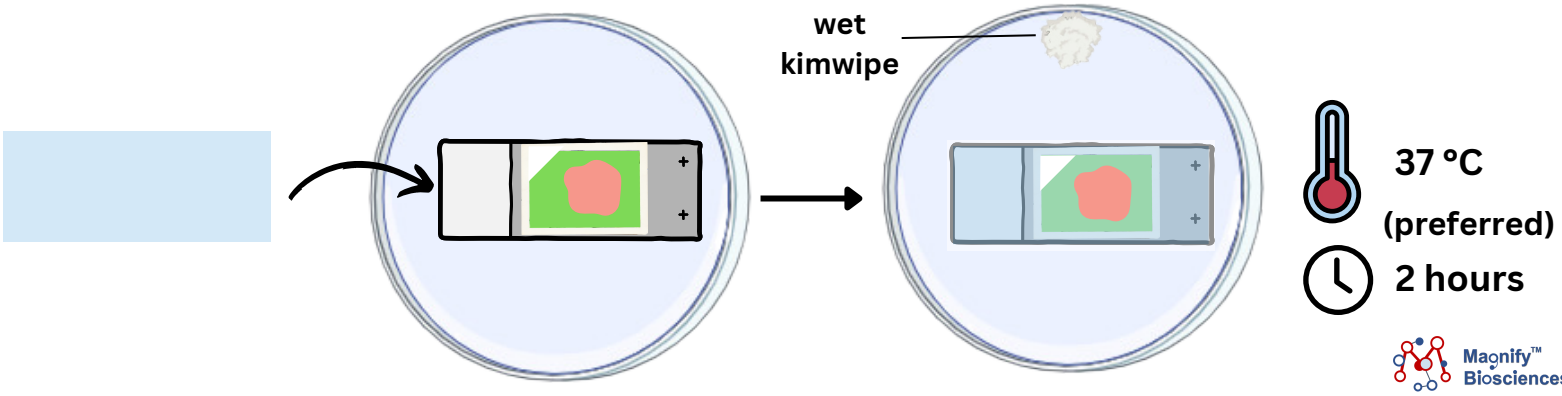
500 uL Solution G in Tube 1 **Activated gelling solution**

Step 5. Fill up the gelling chamber with the Activated Gelling Solution. Add carefully and try not to perturb the tissue. Place the tissue slide in the fridge or on ice/ ice pad and wait for 15 minutes to allow sufficient infiltration. **Caution: Ensure that the activated gelling solution fills the chamber fully or even slightly above the level of the gel chamber (see video demo). It is crucial to prevent incomplete gelling and the formation of bubbles or gaps within the chamber.**



Activated gelling solution

Step 6. **Gelling**: Place the tissue slide in a Petri dish. Cover the slide with the provided plastic cover. Place the wet Kimwipe and close the lid of the Petri dish to create a humid environment. Incubate the slide at 37°C for 2 hours (preferred), OR 45 °C for 1.5 hours. **Caution: It is critical to ensure the plastic sheet cover not drift as it can introduce bubbles.**



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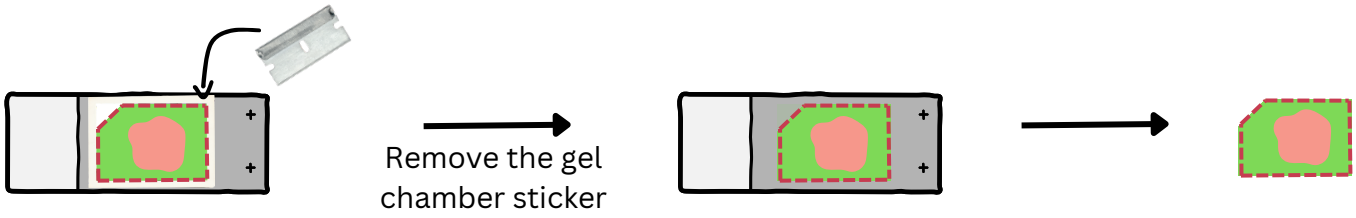
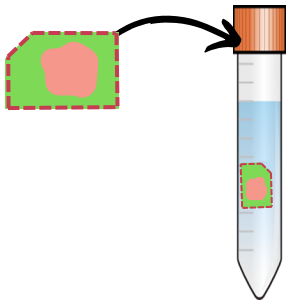
Step 7. **Gel detachment:** Take out the Petri dish from the incubator. For temporary storage before homogenization, store the Petri dish at 4 °C. Remove the plastic sheet cover from the tissue slide. Use a razor blade to cut along the **gel chamber's border** to cut the gel free (see video). Take off the gel chamber sticker and use the blade to clean off any leftover gel outside the chamber. Gently detach the gel with a brush or a gloved finger; it should come off smoothly without dampening it unless it is completely dried.



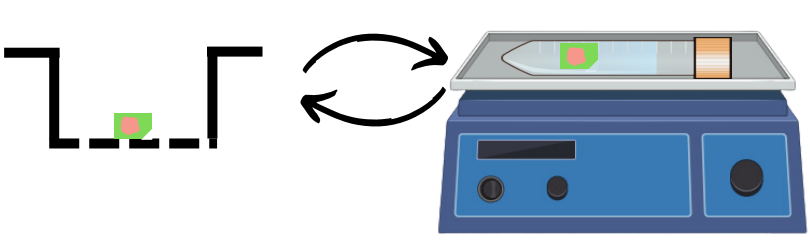
Step 8. **Homogenization**: Carefully transfer the detached gel into a 15 mL Falcon tube (not provided). Add 3 mL of Buffer H, ensure the gel is fully submerged, and incubate at either 95 °C or autoclave at 120 °C, 12 psi (preferred). Incubation time varies depending on tissue type - consult the tissue lookup table for the specifics. If you see precipitates in Buffer H, please warm them to 45 °C for a few minutes, and they will dissolve quickly. **Caution: It is critical to ensure adequate incubation time and temperature for full homogenization. The times listed in the table are just for reference and It might need further optimization. Samples should appear clear after complete homogenization.**



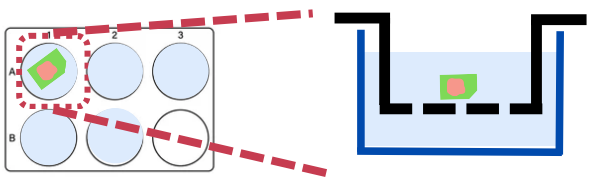
Tissue Type	Homogenization Time	
	95 °C	120 °C, 12 psi
FFPE Optic Nerve	8 Hours	120 Minutes
FFPE Prostate	8 Hours	90 Minutes
FFPE Brain	100 minutes	Not recommended
All Other FFPE	6 Hours	80 Minutes

Step 9. **Buffer Preparation:** Mix 5 mL of 10X Buffer W with 45 mL of water to create 1x Buffer W solution.

Step 10. **Post-homogenization washing:** Place the specimen in the gel catcher to discard the homogenization buffer. For small gelled tissue, use a six-well plate for easier washing. Wash five times with **1X Buffer W** on an orbital shaker at 70 rpm, 15 minutes each, using 5 mL of fresh 1X Buffer W per wash. After washing, gels can be stored at 4 °C in 1X Buffer W or 1X PBS. **Caution: Sufficient washing is vital to remove surfactants and ensure staining quality. For larger gelled tissue, a 15 mL Falcon tube is recommended to provide more space for effective washing.**



OR



Wash on orbital shaker

Wash 5 times @ RT
15 min/wash
5 mL fresh 1X buffer /wash

Step 11. **Staining:** Prepare 1-2 mL of staining solution with labeling reagents of the user's choice using **Buffer S**. For immunostaining, we recommend diluting the antibody or lectin with buffer S to 1 µg/mL and diluting lipid stain and pan-protein stain NHS-ester with buffer S to 2 µg/mL and diluting FISH probes to 10 pM. Incubate the gel with the prepared staining solution in the 15 mL Falcon tube for 1-3 hours at RT. **Caution: The NHS-ester pan-protein stain is known to modify epitopes; therefore, it is advised to apply this stain to the specimen only after all other staining procedures have been completed.**

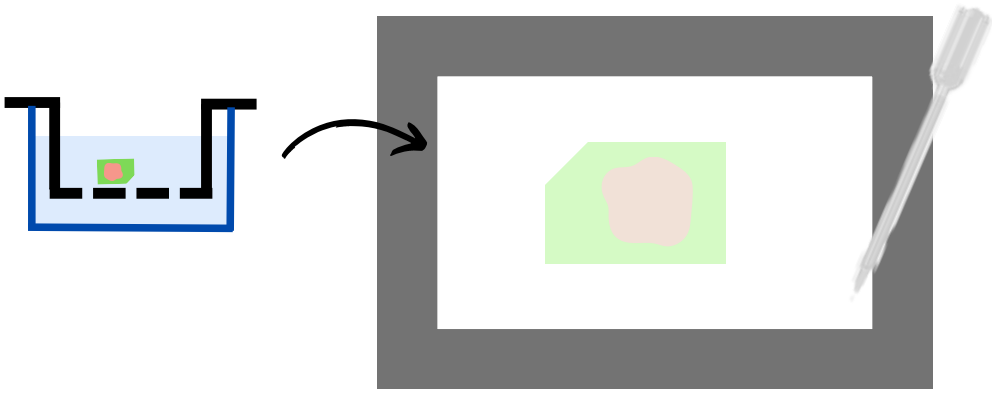
Step 12. **Post-staining washing:** Wash the specimen with 1X Buffer W or 1X PBS again three times, like in step 10. Optionally, User can stain the specimen with DAPI solution.

Direct/Indirect immunostain
Pan-protein stain
lLectin-stain
FISH probe

If secondary staining needed

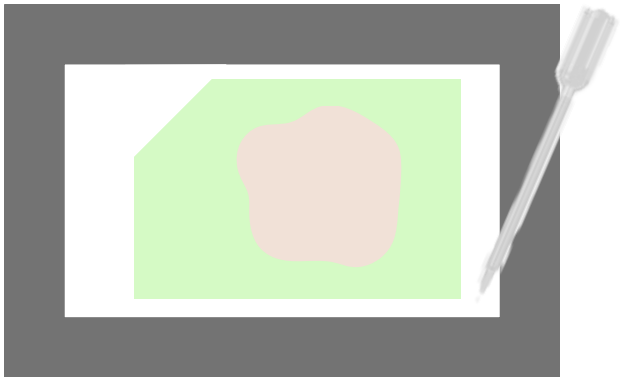
Staining **Post-staining washing**

Step 13. **Expansion:** Transfer the gel to the one-well glass bottom plate, ensuring the orientation of the cut corners as illustrated to keep the objective lens close to the tissue during imaging. If the gel is incorrectly oriented, gently correct it by adding 1 mL of 1X PBS and using the brush to flip it carefully. It's crucial to handle the gel delicately to avoid damage. Fill up the plate with 1X PBS for 4X expansion on the orbital shaker for 15 mins. Use droppers to carefully remove all liquid before imaging.



Alternative orientation

Step 14. For 11X expansion, wash the gel inside the one-well plate with fresh de-ionized water using droppers on the orbital shaker at least three times for 15 minutes each. After the gel has fully expanded, use droppers to carefully remove all liquid before imaging.



Imaging

Step 15. To minimize drifting during imaging, user may optionally coat the sample with a warm 1% agarose solution in ddH2O, ensuring to envelop both the sample's surroundings and its top. Wait for the agarose to solidify fully before starting the imaging process.

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