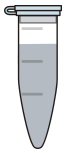
Expansion Microscopy Kit

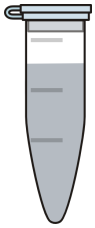
DIRECT PROTEIN ANCHORING VERSION FOR TISSUE

INSTRUCTION MANUAL

CONTENTS

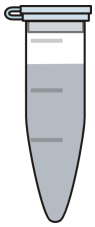


Initiator
Digestion
(20mg)
x6



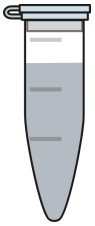
Accelerator

(55µL)



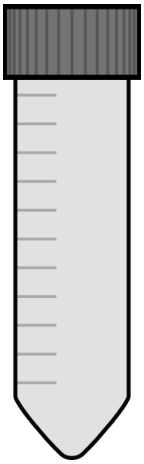
Digestion Enzyme

(200µL)

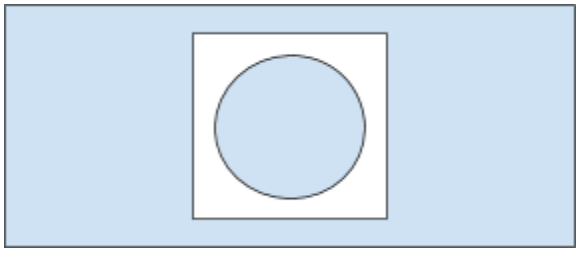


Anchoring

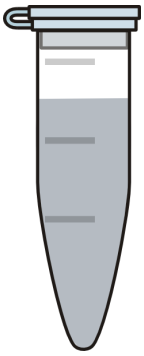
Reagent
(12µL)



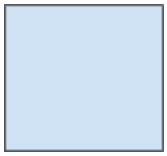
Buffer
(20mL)



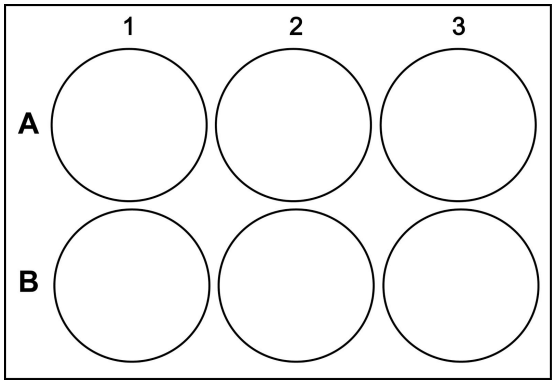
Gelation Plate **x 6**



Monomer Solution
(4 mL)



Coverglass
x 6



6 well imaging plate

Storage

Component Name	**Optimal Storage Temperature**
Digestion Enzyme	- 20 ^{0}C
Digestion Buffer	- 20 ^{0}C
Monomer Solution	- 20 ^{0}C
Accelerator	Room Temperature
Initiator	Room Temperature
Anchoring Reagent	-20 ^{0}C

Prior to use:

Dissolve contents of Initiator in 200 µL of DI water immediately before polymerization step.

Thaw all solutions stored at -20 ^{0}C (see table above), and ensure that no precipitate forms before using. If precipitate forms, vortex the vials briefly to redissolve compounds.

Instructions

Perfusion, Slicing, and Primary Staining (**PRE-KIT**)

Use standard perfusion and slicing followed by primary and secondary antibody staining. Slices of up to 100 µm are compatible with Expansion Microscopy.

We recommend the use of a 24 well plate (not included) for fixation, perfusion, and staining (primary and secondary).

Note: **Expansion Microscopy causes a reduction in fluorescence** (specifically with Cy3, Cy5, and Alexa 647 dyes therefore Secondary Antibodies with these dyes are not recommended). We recommend Secondary Antibodies with Alexa 488, Atto 488, Alexa 594, Atto 647N, CF633, CF405M dyes.

We highly recommend performing a round of sample imaging after secondary antibody staining and **before** initiating sample anchoring.

Sample Anchoring

S1. Dilute 2 µL of the **Anchoring Reagent** with 198 µL of 1X PBS and add to each well of the 24 well plate containing your samples.

S2. Incubate at least 6 hours to overnight (12 to 16 hours) on a rocker at room temperature.

S3. Add 200 µL of 1X PBS to each well containing your samples and place on a rocker for 10 minutes, remove and discard the liquid

S4. Repeat step **S3** two times

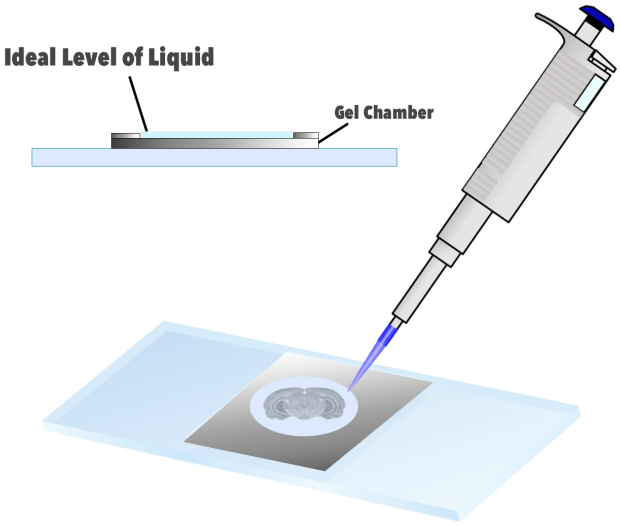
POLYMERIZATION

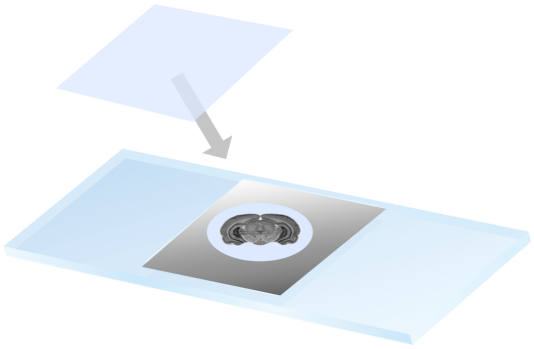
P1. Dilute one of the **Initiator** tubes with 200 µL of DI purified water. Ensure that **Monomer Solution** has been vortexed such that no precipitate forms and solution is not cloudy.

P2. Remove 1X PBS from the wells containing samples and add 200 µL of **Monomer Solution** to each well containing samples. Incubate for 10 minutes on a rocker.

P3. Remove and discard the **Monomer Solution** in the wells.

P4. Add 384 µL of **Monomer Solution** to each well containing samples and allow for 5 minutes of incubation on a rocker. Add 8 µL of **Accelerator**, followed by 8 µL of **Initiator** to each well with **Monomer Solution**, and allow for 1 minute of benchtop incubation.





P5. Transfer the tissue into the gelling chamber of the **Gelation Plate**. Ensure that the sample is not folded and remove excess solution surrounding it. The amount of solution should be even with the height of the gelation chamber.

P6. Gently place a **Coverglass** over the top of the gelation chamber ensuring no air bubbles in the gel.

P7. Let the **Gelation Plate** sit for 2 hours at room temperature out of light to ensure proper polymerization.

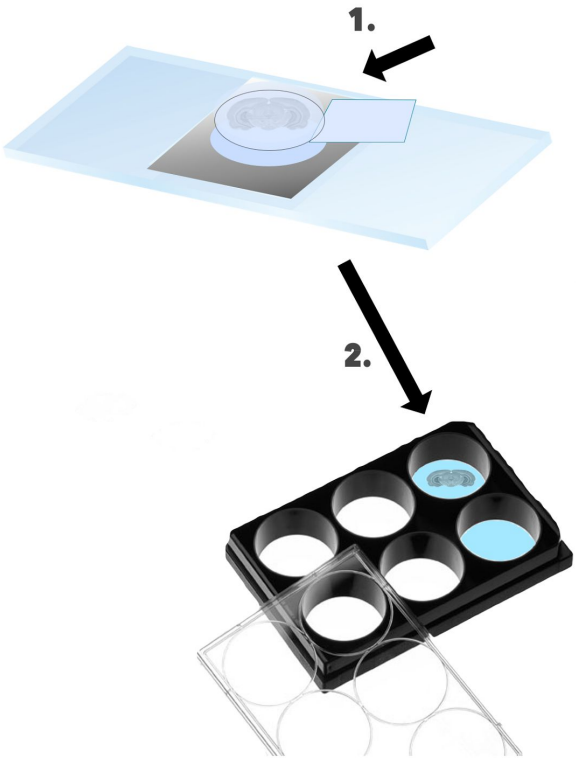
*The redissolved **Initiator** may be discarded after each polymerization is complete.*

DIGESTION AND EXPANSION

D1. Prepare digestion solution by mixing 30 µL of the **Digestion Enzyme** and 2.97 mL of the **Digestion Buffer** in each well of the **6 Well Imaging Plate** which will contain a sample.

D2. Remove the **Coverglass** carefully. The gel will remain adhered to either the **Coverglass** or the **Gelation Plate**.

D3. Carefully, use a spare **Coverglass** to remove the gel from the surface of the **Gelation Plate** or the **Coverglass**, depending on which surface the gel adhered to, as shown below. Place the gelled sample into a well of the **6 Well Imaging Plate** containing the Digestion Solution.



D4. Incubate overnight (18-24 hours) ensuring that the slice is completely submerged.

D5. Wash the hydrogels with 5 mL of ultrapure (cell culture grade) water for 15 minutes

D6. Repeat step D7 twice

D7. Expansion will reach an equilibrium of 4.0X — 4.5X after 4 washes. Remove the water from the plate prior to imaging. Fluorescence Microscopy may now be used to image the expanded samples in the 6 well imaging plate.

ADDITIONAL INFORMATION

The quality of water is **extremely** important. Water of lower purity, containing salt, will result in a lower expansion rate.

Imaging recommendation: For short term imaging, we recommend removing the water from the Expansion Plate so that the gel remains immobile in the plate. For long term imaging, the gel can be embedded in agarose.

TROUBLESHOOTING

The gel is drifting within the well, resulting in difficulties during image capture

Ensure that all water has been removed from the wells before imaging. If the gel continues to move, use 0.8% agarose to embed the gel within the well plate.

The signal-to-noise ratio is very low during imaging or the sample is very dim

Exposure of the sample to free radical polymerization (P7) and expansion (D9) causes reduction in overall sample brightness. Due to variation in the extent of fluorescence reduction, we recommend **not** using Cy3, Cy5, and Alexa 647 fluorophores.

The expansion factor of the gel is below 4x.

The sodium polyacrylate gel must be expanded with ultrapure water (such as cell culture water)