# Ultrastructure Expansion Microscopy (U-ExM)

## 1- Reagents :

Formaldehyde (Sigma 252549)—Ready to use—Store at RT

Acrylamide (AA, 40%, EU0060-A, Euromedex)—Ready to use—Store at 4°C

N,N’-Methylenbisacrylamide (BIS, 2%, EU0560-A, Euromedex)—Ready to use Store  at 4°C

Sodium Acrylate (SA, 97–99%, 408220, SIGMA)

Ammonium persulfate (APS, EU0009-A, Euromedex)

Tetramethylethylenediamine (TEMED, 50406-A, Euromedex)

ddWater  (0,2µM filtered)

Poly-L-Lysine (P8920-100ml, Sigma)—Ready to use—Store at RT

Tween20  ( Sigma P-7949) - Store at RT

Absolute ethanol

Phosphate Buffered Saline (1X and 10X )  (0,2µM filtered)

## 2- Materials :

Forceps home made with plastic cover

Laboratory wipes

Parafilm (M® All-Purpose Laboratory Film)

37 °C incubator

24 - Well cell culture plate (NUNC)

6 - well cell culture plate (NUNC)

Microcentrifuge tubes (1.5 mL)

Vortex

Shaker

Thermoblock

Razor blade

12 mm round coverslips (#1.5 Thickness (0.17 mm or 0.0067 in.)

Glass beaker (1 per expansion)

ibidi chambers (#80156) or Olympus Metal Dish Chambers( RA-35-18-2000-06) with 24 mm coverslips.

## 3- Solutions :

### Formaldehyde / acrylamide (FA/AA) mix

|  |  |  |
| --- | --- | --- |
| Product | Volume | Final concentration |
| Formaldehyde 37% | 19 µL | 0,7% |
| Acrylamide 40% | 25 µL | 1% |
| PBS 1X | 956 µL |  |

### Sodium acrylate stock solution (SA)

Prepare 38% (w/w) stock solution

Dissolve little by little 19 g of sodium acrylate into 31 mL dd water (31g) on ice.

Store at 4°C after dissolution.

### U-ExM Monomer solution (MS)

|  |  |  |  |
| --- | --- | --- | --- |
| Reagent | [Stock solution] | Volume (µL) | [Final] |
| Sodium acrylate (SA) | 38% (w/w) | 500 | 23 % w/v |
| Acrylamide (AA) | 40 % | 250 | 10% w/v |
| Bis-acrylamide (BIS) | 2% | 50 | 0,1% w/v |
| PBS | 10X | 100 | 1X |
| Total |  | 900 µL |  |

Make 90 µL aliquots and store at -20°C for up to 2-3 weeks maximum. Note that the solution will not freeze.

### Denaturation buffer

|  |  |  |  |
| --- | --- | --- | --- |
| Reagent | [Stock solution] | Volume / weight | [Final] |
| SDS | 20% (694 mM) | 28,82 mL | 200 mM |
| NaCl | 5M | 4 mL | 200 mM |
| Tris-BASE | - | 0,6 g | 50 mM |
| dd H2O | - | 10 mL |  |
| HCl | - | pH to 9,0 |  |
| dd H2O |  | Fill up to 100 mL |  |

# Method :

## Day 0 :

### MS preparation

Prepare MS at least the day before the UexM.

### Cell culture

Prepare cells to have about 4x106 cells per 12mm coverslip.

## Day 1 :

### Cell preparation

Load 12mm coverslip into wells of a 24 wells plate.

Cover the first coverslip with 200µL of Poly L Lysine then recover the liquid and cover the second coverslip etc…

Leave the coverslip to dry for 10 minutes.

Wash your cells as usual and load 250µL of cells at about 1.6x107/mL

Leave them to seat for 5-10 minutes (check under microscope the repartition)

### Activation

Remove the excess of cells suspension and add 1mL of FA/AA solution for 4h at 37°C (or ON at +4°C) with slow agitation.

Fill up the empty wells with water to avoid evaporation.

### Gel polymerisation

Prepare a 24 wells plate lid on ice with parafilm. (leave it to cool down)

Put the coverslip on a 35µL drop of cold MS solution (facing down)

Add to 90µL of the cold MS solution :

* 5µL of cold 10% TEMED
* 5µL of cold 10% APS

Vortex few seconds

Replace the tube on ice

Put 35µL per coverslip of MS+TEMED+APS on the parafilm on ice.

Immediatly put the coverslip on top of this drop (facing down)

Incubate 5 minutes on ice and then 30 minutes at 37°C

### Denaturation

After the polymerisation, remove the coverslip from the parafilm and put the coverslip + gel into a well of a 6 wells plate filled with 1mL of denaturation buffer.

Incubate 15 minutes at room temp with moderate agitation

Prewarm 1.5 mL tubes with 1mL of denaturation buffer at 95°C in a thermoblock.

Using home made forceps (made with plastic cover), transfer the gel into a tube with 1 mL of prewarmed denaturation buffer.

Fill up the tube with extra denaturation buffer if needed.

Incubate in the thermoblock 30 minutes at 95°C.

### First expansion

Put each gel into a glass beacher with 100 mL of dd water.

Incubate for 30 minutes

Repeat twice.

Measure the diameter of the gel to estimate the expansion factor.

You can stop at this step and leave the gel into a petri dish with 15mL of water overnight at 4°C.

## J2

### Antibody labelling

Remove the water and load 100mL of 1X PBS, incubate for 10 minutes

Repeat twice.

Put the gel in a well of a 6 wells plate.

Cut a piece of the gel (diameter of 8 mm = inner diameter of the top of 1mL tip)

Put the piece of gel into a well of a 24 wells plate (cytoskeletons facing up) and load 250 µL of PBS on it.

Remove PBS and put 250µL of diluted primary antibodies on the gel (antibodies should be twice concentrated compare to normal IFA).

Incubate 2 hours at room temp in a moist chamber with slow agitation.

Wash 3 times in 100µL of 1X PBS

Since now, you must incubate your gels into the dark until the end of UexM.

Put 250µL of diluted secondary antibodies on the gel (same as primary : twice concentrated compare to normal IFA)

Incubate 2 hours at room temp in a dark moist chamber with slow agitation.

Incubate with DAPI 10µg/mL in PBS for 10 minutes in a dark moist chamber with slow agitation

Wash 3 times in 100µL of 1X PBS in a dark moist chamber with slow agitation

### Second expansion

Put the gel in a well of a 6 wells plate with 5 mL dd water.

Incubate 30 minutes in the dark

Exchange the water with 5 mL dd water and re-incubate for 30 minutes in the dark

Repeat this step and incubate overnight in the dark to reach the final expansion.

Store protected from light.

Add Sodium azide to a 0,02% final concentration as preservative.