Whole mount immunofluorescence on embryonic tissue slices

(adapted from Gómez-Gaviro et al., 2017)

DAY 1

1) **Fix embryonic tissue slices (~200-400µm in thickness) in formalin**
    a. Incubate the samples in 10% formalin O/N at RT
    b. Wash in PBST 5'X3

    Note: Prepare 500ml PBST (50 ml 10xPBS + 0.5 ml Tween 20 + 449.5 ml dH_20)

DAY 2

2) **Reagent 1 ****LIGHT SENSITIVE******
    a. Prepare Reagent 1 in 50 ml tube, andshake until dissolved:

i.	Distilled water	14 ml
ii.	Urea	10 g
iii.	N, N, N, N – tetrakis ethylenediamine	10 ml
iv.	Triton	6 ml
v.	DAPI	1:5000

    b. Incubate the samples in Reagent1 O/N at 37^0C, rotating. Make sure the samples are fully immersed in Reagent 1 (depends on the size of the sample)
    c. Wash in PBST 5'X3

3) **Antigen retrieval (Heat-based antigen retrieval) ****LIGHT SENSITIVE******
    a. Preheat the steamer

b. Preheat the antigen unmasking working solution in a microwave for 10 mins or by heating the solution over a Bunsen burner until boiling

   Note: prepare 450ml antigen unmasking working solution (4.2 ml antigen unmasking stock solution + 450 ml dH_20)

c. Transfer the boiled antigen unmasking solution to the steamer and steam the samples in the antigen unmasking solution for ~10-15mins

d. After steaming is complete, transfer the samples to FRESH 1XPBS solution

4) Block unspecific staining ****LIGHT SENSITIVE****

a. Incubate samples in blocking buffer for 4 hours at 37^0C, rotating. Make sure the samples are fully immersed in blocking buffer (depends on the size of the sample)

b. Proceed directly to step 5. **DO NOT WASH**

5) Primary antibody ****LIGHT SENSITIVE****

a. Dilute the primary antibody in blocking buffer (concentration depends on the antibody used)

b. Incubate the samples with primary antibody 4 hours at 37^0C, rotating. Make sure the samples are fully immersed in primary antibody (depends on the size of the samples)

c. Wash in PBST 5'X3

   Note: Prepare 500ml PBST (50 ml 10xPBS + 0.5 ml Tween20 + 449.5 ml dH_20)

6) Secondary antibody ****LIGHT SENSITIVE****

a. Dilute the secondary antibody in blocking buffer (1:200)

b. Incubate the samples with the secondary antibody for 4 hours at 37^0C, rotating. Make sure the samples are fully immersed in secondary antibody (depends on the size of the sample)

c. Wash the slides in PBST 5'X3

7) Reagent 2

a. Prepare Reagent 2, shake in 50 ml tube until dissolved:

i.	Distilled water	1.5 ml
ii.	Urea	2.5 g
iii.	Sucrose	5 g
iv.	Triethanolamine	1 ml

b. Incubate the samples in Reagent2 O/N at 37^0C, rotating

c. Mount the samples for imaging

Reference:

Gómez-Gaviro, M.V., Balaban, E., Bocancea, D., Lorrio, M.T., Pompeiano, M., Desco, M., Ripoll, J., and Vaquero, J.J. (2017). Optimized CUBIC protocol for three-dimensional imaging of chicken embryos at single-cell resolution. Development *144*, 2092-2097.