Immunofluorescence Labeling Protocol for 3D Cell Culture

STEP		TEMPERATURE	TIME*
1	Warm-up Fixative (4% Paraformaldehyde), PBS**-Cello™-IF3-Primary Antibody Solution-Secondary Antibody Solution ***-Mounting Medium****	37 °C	10 min
2	Aspirate medium, Fix the sample **while in the gel**	37 °C	30 min-1 hr
3	Incubate with Cello™-IF3	37 °C	3X, 10 min
4	Incubate with Primary Antibody in Cello™-IF3	37 °C	1-2 hr
5	Incubate with Cello™-IF	37 °C	3X, 10 min
6	Incubate with Secondary Antibody in Cello™-IF3, Keep in dark	37 °C	30 min-1,5 hr
7	Incubate with PBS , Keep in dark	37 °C	3X, 10 min
8	Cover with Mounting Medium, Keep in dark	RT	
	Close the lid tightly, Keep in dark until examination.	4 °C	
			TOTAL : 5-6 hr

*Timings are for immunolabeling of organoids or spheroids. Durations for each steps can be adjusted according to the size of organoids/spheroids and the gel.
**PBS: Phosphate Buffered Saline
***Dilute primary and secondary antibodies in CellO-IF.
***Mounting Medium:1,2 uL Hoechst or DAPI+675 uL PBS + 675 uL Glycerol

TIPS:
-Using a cell culture container with high optical quality is highly recommended (e.g., glass-bottomed dishes and well-plates, multi-chambered slides, CellO ™ - M). The container here represents CellO ™ - M.
-Low-speed orbital shaker can be used to facilitate penetration.
-Most samples are examined on the same day,. Nuclear staining improves after 24 hours in some thick samples.
-Start with the validated dilution rates for each antibody. Adjustment of the dilution rates may be needed based on the nature of the sample and the gel.
-The same protocol can be used for the samples not embedded in the gel.