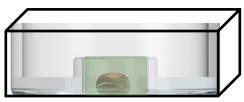
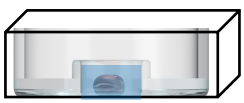
CELLORAMA™

Immunofluorescence Labeling Protocol for 3D Cell Culture

	STEP		TEMPERATURE	TIME*
1	Warm-up Fixative (4% Paraformaldehyde), PBS**, Cello™-IF, Primary Antibody Solution, Secondary Antibody Solution , and Mounting Medium***		37 °C	10 min
2		Aspirate medium, Fix the sample **while in the gel**	37 °C	1 hr
3		Incubate with Cello™-IF	37 °C	3X, 10 min
4		Incubate with Primary Antibody Solution in Cello™-IF	37 °C	1,5-2 hr
5		Incubate with Cello™-IF	37 °C	3X, 10 min
6		Incubate with Secondary Antibody Solution in Cello™-IF Keep in dark	37 °C	1-1,5 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

*That flowchart describes timings for the immunolabeling of organoids or spheroids.
**PBS: Phosphate Buffered Saline
***Mounting Medium:1,2 uL Hoechst or DAPI+675 uL PBS + 675 uL Glycerol

NOTES:
-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.
-The same protocol can be used for the samples in the medium, not embedded in the gel.
-Low-speed orbital shaker can be used if the size of the 3D-growing sample is large.
-More details about the protocol are available in the CellO™-IF User Guide.