

Three-dimensional histochemistry and imaging of human gingiva

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BABB protocol

Day 1 (6 h)

Dehydration		
Wash in PBS	2x 60 min	R.T.
Incubate in 50% methanol in PBS	50 min	R.T.
Incubate in 80% methanol in PBS	50 min	R.T.
Incubate in 100% methanol	2x 30 min	R.T.
Bleaching		
Bleach in 1 part 30% H ₂ O ₂ , 1 part DMSO and 4 parts methanol at 4° C	O/N	4°C

Day 2 (7 h)

Rehydration		
Incubate in 100% methanol	3x 30 min	R.T.
Incubate in 20% DMSO in methanol	1x 30 min, 1x 60 min	R.T.
Incubate in 80% methanol in PBS	50 min	R.T.
Incubate in 50% methanol in PBS	50 min	R.T.
Wash in PBS	2x 45 min	R.T.
Incubate in PBS/0.2% Triton X-100	50 min	R.T.
Blocking of nonspecific staining		
Incubate in PBS/5% BSA	O/N	R.T.

Day 3-8 (1 h)

Immunolabeling		
Incubate in solution with primary antibodies in PBS/0.2% Tween-20	5 days	R.T.

Day 9 (2 h)

Immunolabeling		
Wash in PBS	3x 30 min	R.T.
Incubate in solution with secondary antibodies in PBS/0.2% Tween-20	2 days	R.T.

Day 10 (8 h)

Wash in PBS/0.2% Tween-20	4x 45 min	R.T.
Dehydration		
Incubate in 50% methanol/H ₂ O	50 min	R.T.
Incubate in 70% methanol/H ₂ O	50 min	R.T.
Incubate in 80% methanol/H ₂ O	45 min	R.T.
Incubate in 96% methanol/H ₂ O	45 min	R.T.
Incubate in 100% methanol	3x 30 min	R.T.
Incubate in 50% BABB/50% methanol	O/N	R.T.

Day 11 (1 h)

Clearing		
Incubate in BABB (2 parts benzylbenzoate, 1 part benzylalcohol)	Until sample is clear (30-60 min)	R.T.