

Immunohistochemistry (IHC) for paraffin and frozen tissue sections

Ver1.3

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Introduction:

The IHC protocols for paraffin and frozen tissue sections described here assume the user has basic understanding and familiarity with immunocytochemical procedures, in particular the fixation, processing and cutting of tissue sections for either application. This IHC protocol provides a list of mAbs validated for use in IHC (Tables 1 and 2) along with mAb-specific information needed for successful IHC (antigen retrieval conditions, antibody dilutions, recommended detection kits, etc.).

The detailed, mAb-specific information and the representative images shown here were kindly provided by Drs. N. Lax, L. Greaves, J. Murphy, and D. Turnbull, Mitochondrial Research Group, Newcastle University.

MitoSciences Mitochondrial Monoclonal Antibodies for Immunohistochemistry

Table 1

Tissue type	Primary antibody	MitoSciences Catalog #	Fixative	Antigen retrieval	Antibody dilution	Antibody incubation	Method of detection	Quality of staining
Muscle	Porin	MSA05	Snap Frozen in liquid nitrogen cooled isopentane, then cryostat sections post-fixed in 4% PFA	Not required	1:2000	1 hour	Primary antibodies were detected with a polymeric labeling two-step method from Menapath (MP-XCPDAB-U100). In this procedure, all primary antibodies are labeled with a universal probe (which recognizes antibodies raised in both mouse and rabbit species) for 30 minutes and a HRP-polymer probe for 30 minutes.	****
	Complex II 70 KDa	MS204	Snap Frozen in liquid nitrogen cooled isopentane, then cryostat sections post-fixed in 4% PFA	Not required	1:1000	1 hour		****
	Complex I 20 KDa, NDUFB8	MS#105	Snap Frozen in liquid nitrogen cooled isopentane, then cryostat sections post-fixed in 4% PFA	Not required	1:100	1 hour		****
	Complex IV-subunit-I	MS404	Snap Frozen in liquid nitrogen cooled isopentane, then cryostat sections post-fixed in 4% PFA	Not required	1:400	1 hour		****
	Complex IV-subunit-IV	MS408	Snap Frozen in liquid nitrogen cooled isopentane, then cryostat sections post-fixed in 4% PFA	Not required	1:800	1 hour		****
Colon	Complex I 30 KDa, NDUFS3	MS110	Formalin fixed, paraffin-embedded	Not required	1:100	1 hour		****
	Complex II 30 KDa, Ip	MS203	Formalin fixed, paraffin-embedded	Not required	1:200	1 hour		****
	Complex III core 2	MS304	Formalin fixed, paraffin-embedded	Not required	1:500	1 hour		****
	Complex IV subunit I	MS404	Formalin fixed, paraffin-embedded	Not required	1:200	1 hour		****
	Complex IV subunit IV	MS408	Formalin fixed, paraffin-embedded	Not required	1:200	1 hour		****
	Complex V - OSCP	MS505	Formalin fixed, paraffin-embedded	Not required	1:500	1 hour		**
CNS	Porin	MSA05	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:10000	1 hour		****
	Complex I 20 KDa, NDUFB8	MS105	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:300	1 hour		****
	Complex I 30 KDa, NDUFS3	MS110	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	1:1000	1 hour		****
	Complex I 39 KDa, NDUF9	MS111	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	1:100	1 hour	**	
	Complex I 30 KDa, NDUFS3	MS110	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	1:1500	1 hour	*	
	Complex IV-subunit-I	MS404	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	1:10000	1 hour	****	
	Complex IV-subunit-IV	MS408	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	1:8000	1 hour	****	

MitoSciences Mitochondrial Monoclonal Antibodies for Immunohistochemistry

Table 2

Tissue type	Primary antibody	MitoSciences Catalog #	Fixative	Antigen retrieval	Antibody dilution	Antibody incubation	Method of detection	Quality of staining
CNS	SOD2	NA- 6H7BH7	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:1,000*	1 hour	Primary antibodies were detected with a polymeric labeling two-step method from Menapath (MP-XCPDAB-U100). In this procedure, all primary antibodies are labeled with a universal probe (which recognizes antibodies raised in both mouse and rabbit species) for 30 minutes and a HRP-polymer probe for 30 minutes.	****
	SOD2	MS732- (9E2BD2)	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:100	1 hour		****
	TFP	MS734- 4A8BG12	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:100	1 hour		****
	MCAD	NA-9D9AG8	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	ND	1 hour		***
	MCAD	MS726- 3B7BH7	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:1,1000	1 hour		****
	ACAT1	MS718- 9H10AB4	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:100	1 hour		**
	NNT	MS701- 8B4BB10	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:100	1 hour		***
	NNT	NA-3B8BC8	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:100 (weak)	1 hour		***
	VCLAD	MS707- 6A9AF2	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:250	1 hour		***
	MITOFILIN	MSM02- 2E4AD5	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	1:100	1 hour		****
	DECR1	MS711- 8B1AD10	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	ND	1 hour		***
	FUMERASE	NA-4A4BG5	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	ND	1 hour		**
	FUMERASE	MS709-	Formalin fixed, paraffin-embedded	1 min pressure cook 1mmol EDTA pH8	ND	1 hour		**

MitoSciences Mitochondrial Monoclonal Antibodies for Immunohistochemistry

		8F12BB5						
SCHAD	MS706-1A12BC8	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	1:250	1 hour		**	
CYCLOPHILIN	MSA04-E11AE12BD4	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	>1:1,000	1 hour		****	
PDH E2/E3	MSP06-13G2AE2BH5	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	1:1,000	1 hour		****	
PDH E2	MSP05-15D3G9C11	Formalin fixed, paraffin embedded	1 min pressure cook 1mmol EDTA pH8	1:1,000	1 hour		****	
	NA = not in the catalog but available on special order							

OXPHOS Subunit Immunohistochemistry for Paraffin Sections (Human Brain)

1. Fix in formalin, then embed in paraffin.
2. Deparaffinise and rehydrate sections in histoclear and graded alcohols
3. Rinse in dH₂O
4. Heat-Induced Antigen Retrieval: Pressure cook sections in 1 mmol EDTA pH 8, e.g., using Menarini Decloaker set to reach a temperature of 124.5 degrees C for 1 min and a pressure of approx 15-17 psi then run through a cooling cycle
5. Rinse in dH₂O, block endogenous peroxide in 3% hydrogen peroxide for 20 minutes
6. Wash sections in x2 TBS and a final wash in TBST
7. Incubate sections in primary antibody at room temperature for 1 hour
8. Wash sections in TBS 2x5 min
9. Wash in TBST 1x5min
10. Incubate sections in Universal probe (yellow reagent) for 30 minutes
11. Wash sections in TBS 2x5 min
12. Wash in TBST 1x5min
13. Incubate in HRP-Polymer Reagent (orange reagent) for 30 minutes
14. Wash 3x5min in TBS
15. Make up DAB using the ratio of 1ml of DAB substrate to 1 drop of liquid DAB. Make up just prior to use and pipette onto slides, incubate for 2-4 minutes
16. Wash in tap water
17. Counterstain in Meyers Haematoxylin for 5 minutes
18. Wash in tap water
19. Dehydrate in EtOH, clear in 2 changes of Histoclear, mount in DPX

NOTE: Primary antibodies are detected with a polymeric two-step labelling method available from

Menapath, X-Cell-Plus HRP Detection with DAB - product code: MP-XCPDAB-U100

http://www.menapath.co.uk/index.php?page_id=8

OXPHOS Subunit Immunohistochemistry for Fixed Frozen Sections (Human Muscle)

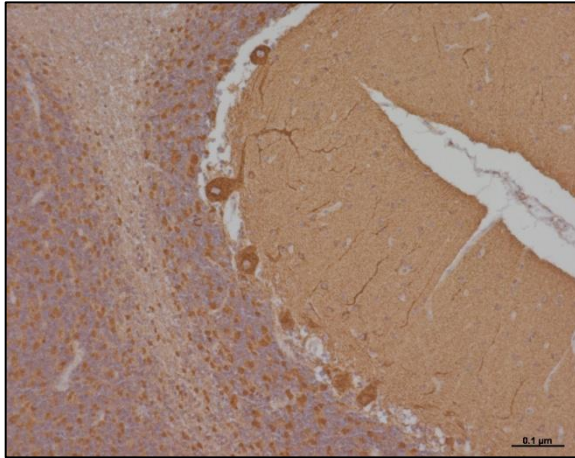
1. Snap-freeze tissues in liquid nitrogen-cooled isopentane and store at -80°C .
2. Cut cryostat sections and then fix the sections in in 4% paraformaldehyde in 0.1M phosphate buffer for 10 min at 4°C
3. Rinse in dH_2O
4. Wash sections in TBS containing 0.1% Tween 20 (TBST) for 10 min
5. Permeabilise sections and inhibit endogenous peroxidase activity in a graded methanol series
 - 70% (v/v) for 10 minutes
 - 95% (v/v) + 0.3% (v/v) H_2O_2 for 10 minutes
 - Absolute methanol for 20 minutes
 - 95% (v/v) + 0.3% (v/v) H_2O_2 for 10 minutes
 - 70% (v/v) for 10 minutes
 - TBST for 5 minutes
6. Incubate sections in primary antibody at room temperature for 1 hour. Antibodies are diluted in 4% BSA in TBST
7. Wash sections in TBS 2x5 min
8. Wash in TBST 1x5min
9. Incubate sections in Universal probe (yellow reagent) for 30 minutes
10. Wash sections in TBS 2x5 min
11. Wash in TBST 1x5min
12. Incubate in HRP-Polymer Reagent (orange reagent) for 30 minutes
13. Wash 3x5min in TBS
14. Make up DAB using the ratio of 1ml of DAB substrate to 1 drop of liquid DAB. Make up just prior to use and pipette onto slides, incubate for 2-4 minutes
15. Wash in tap water
16. Counterstain in Meyers Haematoxylin for 15s
17. Wash in tap water
18. Dehydrate in EtOH, clear in 2 changes of HistoClear, mount in DPX

NOTE: Primary antibodies are detected with a polymeric two-step labelling method available from

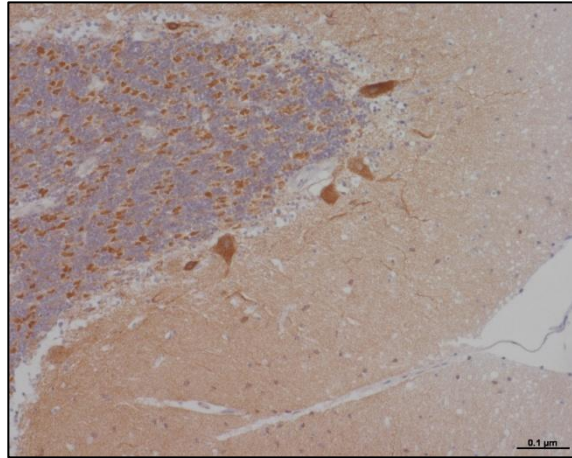
Menapath, X-Cell-Plus HRP Detection with DAB - product code: MP-XCPDAB-U100

http://www.menapath.co.uk/index.php?page_id=8

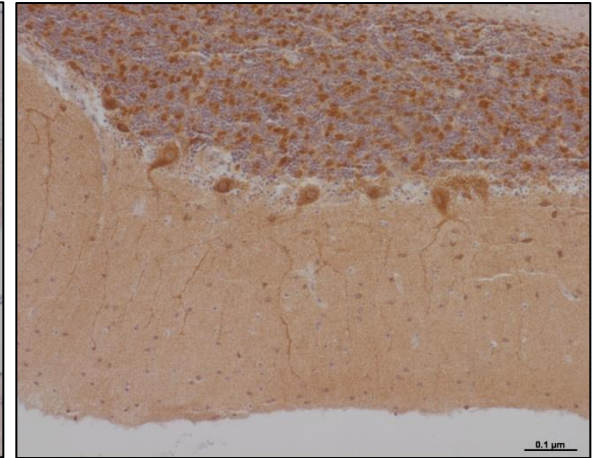
Cerebellum immunohistochemistry – Paraffin tissue sections from control human brain Kindly provided by Dr. N. Lax and D. Turnbull, Mitochondrial Research Group, Newcastle University.



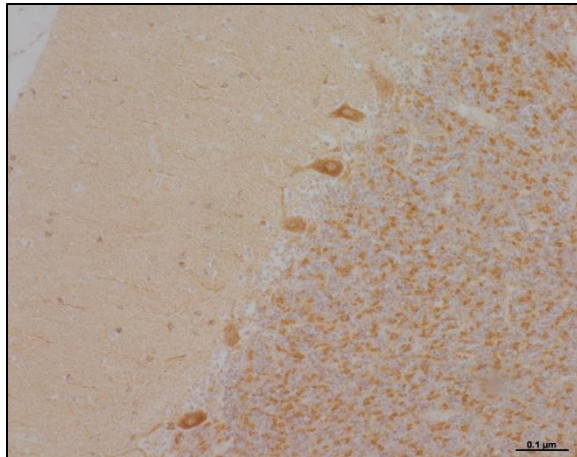
Porin



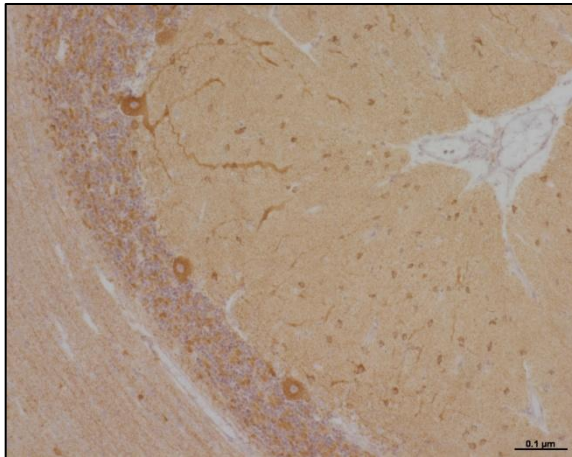
Complex I 20 KDa



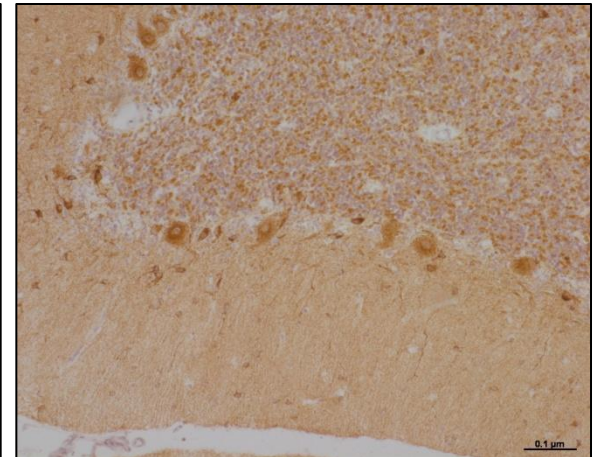
Complex I 30 KDa



Complex I 39 KDa



Complex IV subunit I

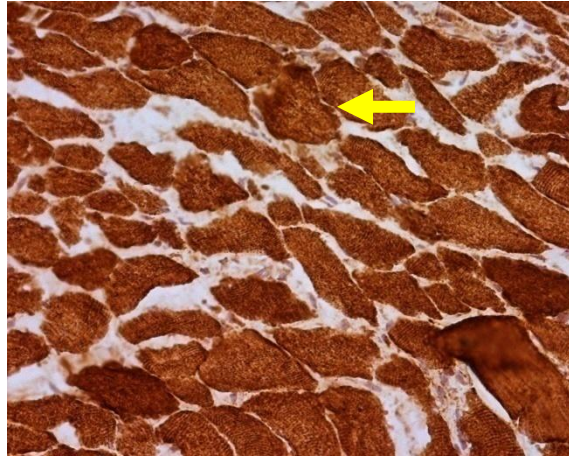


Complex IV subunit IV

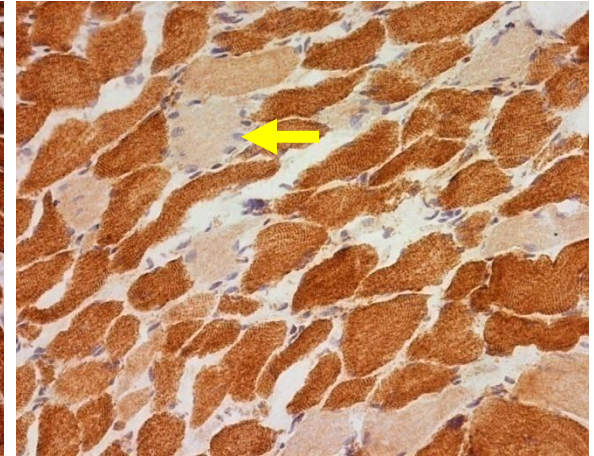
Skeletal Muscle Immunohistochemistry – Fixed Frozen Tissue sections from a patient with a single large deletion of the mtDNA. Kindly provided by Dr. J. Murphy and D. Turnbull, Mitochondrial Research Group, Newcastle University.



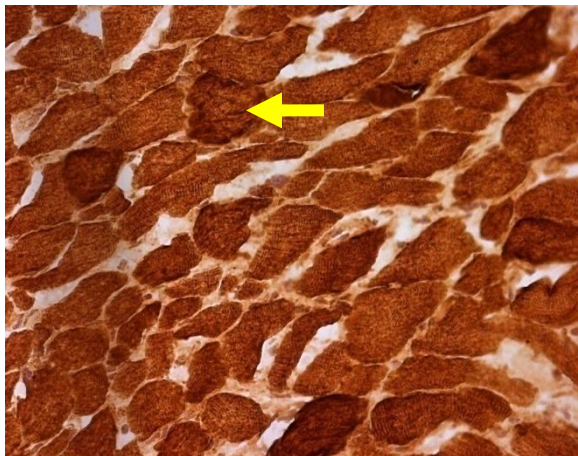
H&E



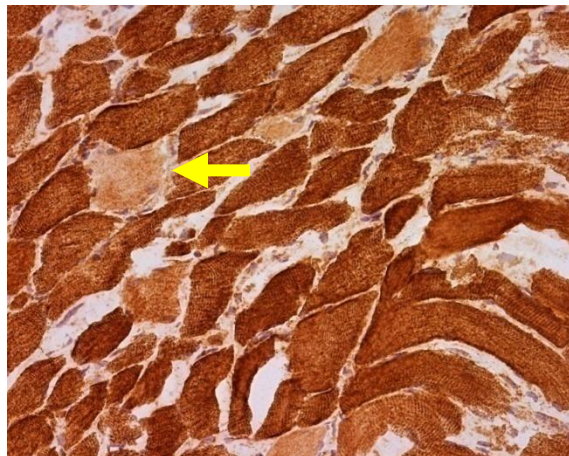
Porin, MSA05



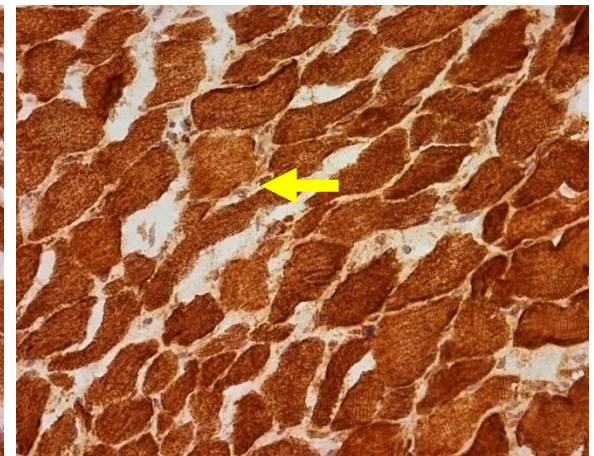
Complex I, 20 KDa NDUFB8, MS105



Complex II ,70 KDa Fp, MS204

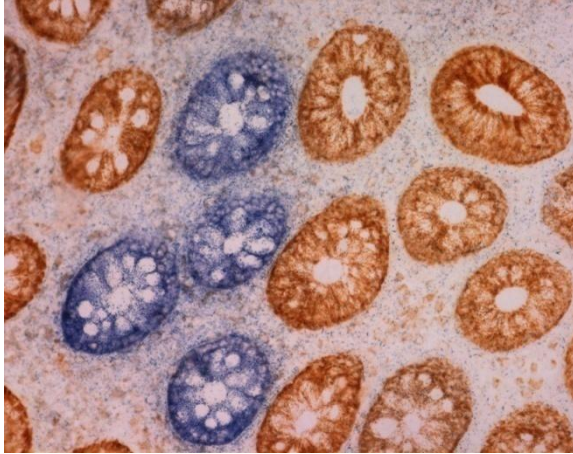


Complex IV, subunit I, MS404

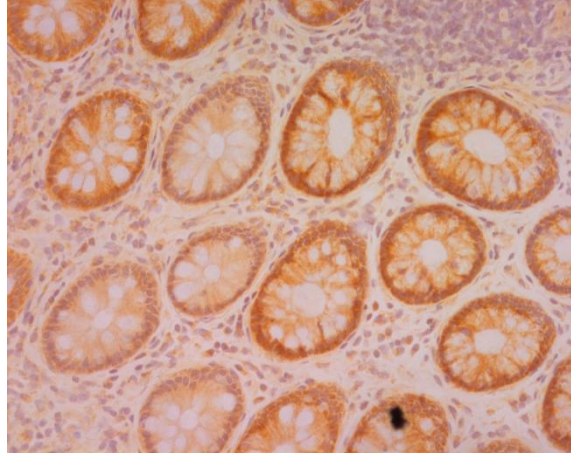


Complex IV, subunit IV, MS408

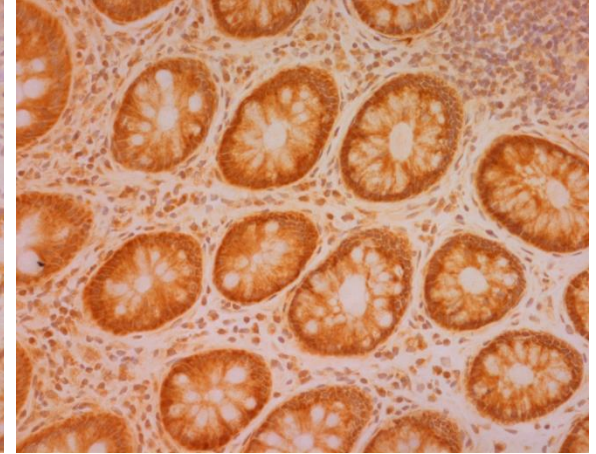
Colon immunohistochemistry – Tissue sections from a normal ageing patient. Tissues snap-frozen in liquid nitrogen cooled isopentane, and frozen sections post-fixed in 4% paraformaldehyde. Kindly provided by Dr. L. Greaves and D. Turnbull, Mitochondrial Research Group, Newcastle University. For more details, see Taylor et al., J. Clin. Invest. 112:1351-1360 (2003)



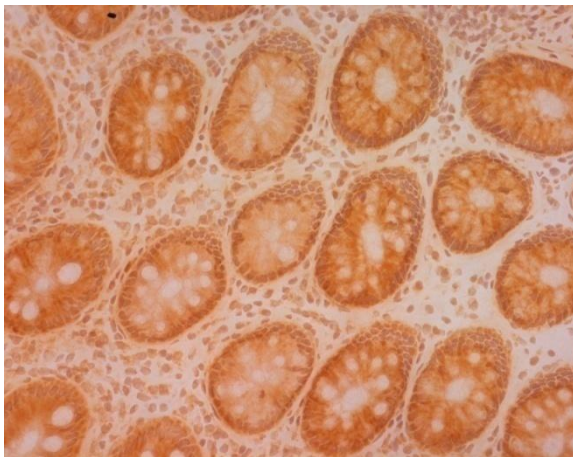
COX/SDH (enzyme activity histochemistry)



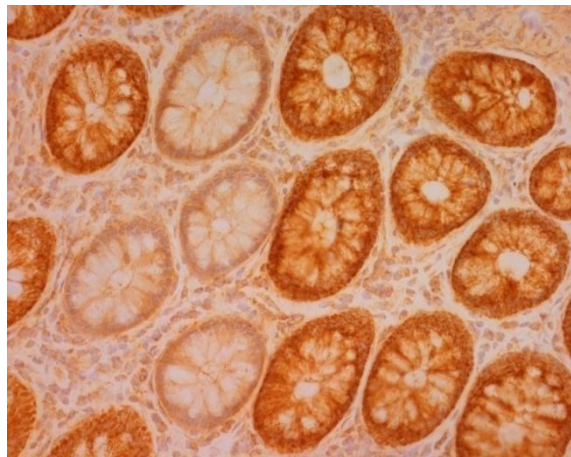
Complex I, 30 kDa NDUF53, MS110



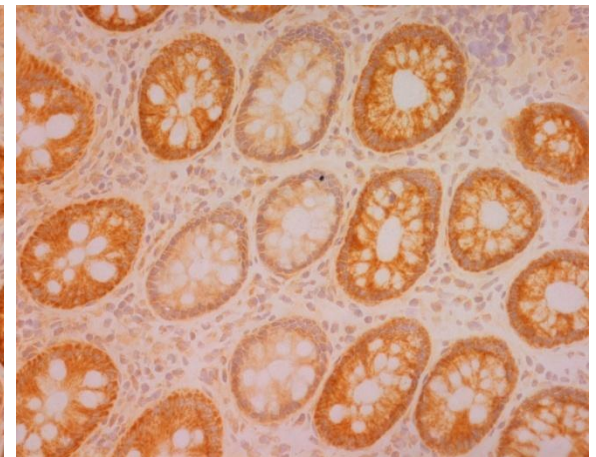
Complex II, 30 kDa Ip, MS203



Complex III, Core 2, MS304



Complex I, subunit-1, MS404



Complex IV, subunit-4, MS408