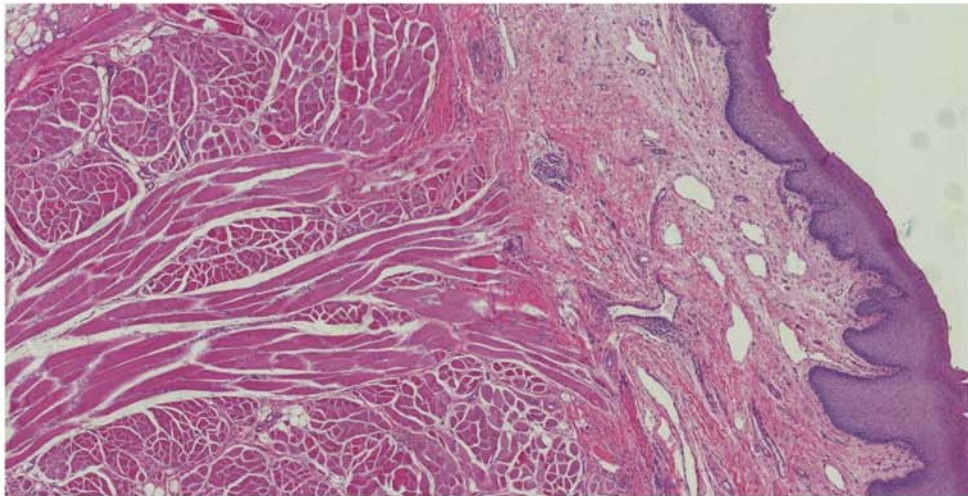


OPERATING MANUAL

GREENFIX

NOT TOXIC FORMALIN SUBSTITUTE



Replace the formalin in your laboratory?

NOW IT IS POSSIBLE!!

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1.0 Preface

In the last 20 years lots of laboratories tried to substitute the formalin with other less toxic fixatives; but the results were not so much satisfactory: in morphology and in IHC method answers took place lot of changes.

But why change the formalin?

In Pathology Anatomy laboratory the formalin is used in large quantity and its dangerousness is usually underestimated: technicians and pathologists actually are exposed to formaldehyde diluted solution. We have to consider that the exposition is daily and frequently; for this reason it is important to attach the right weight to the chemical risk of formalin as carcinogenic agent.

Up to now lot of technicians and pathologists still have doubts concerning formalin dangerousness, but we remark that much relative articles have been yet published.

In 1996 the formaldehyde has been classified as 3 Category carcinogenic substance by the European Community¹(receipt in Italy the following year) and on the label must be appear the R phrases number 40 (Limited evidence of a carcinogenic effect).

In 2004 the IARC² has published a study³ where the formalin chemical agent has been classified as "carcinogenic for human body" (class 1). The researches it was highlighted that the formalin provokes nose-pharyngeal cancers. Available data have also shown that there is a limited association with the nose cavity and the paranasal sinuses, and strong but not enough proofs for leukaemia development.

In 2003⁴ an American study has evaluated formalin exposition effects on 25,619 factory people from 1994 to 1996. The results have demonstrated that these workers (exposed to formaldehyde high levels) have a 3.5 risks more than people with low levels to check leukaemia. Another article lately published on Toxicology⁵ has shown a relation between gene changes and chronic exposition to formalin concerning workers in pathology anatomy labs.

For example, we remark that in accordance with the Italian Regulatory the 81st ordinance 2008 concerning health and safety protection in working place (article 15) establishes that for operator's safety, a hazardous product must be replaced by another not or less hazardous.

For this reason replace formalin in Anatomy Pathology Laboratory is a priority more and more urgent. To satisfy this need; in 2007 Diapath has create a new, not toxic and not harmful formulation that does not involve relevant changes to formalin fixation protocols. During all 2008 different tests were performed in Italian Centres creating the final formulation of **GreenFix** and **GreenFix Plus**.

Greenfix is an ethandial and alcohol based fixative with the add of preserving principles for morphology and tissue antigenicity. This new formulation allows to have a better visualization of nuclear chromatic detail.

The ethandial is a bialdehydic molecule with an action process as formaldehyde⁷, it entails a lower impact on protocols change and preparations reading.

The obtained results have shown that **GreenFix** is a universal formalin substitute fixative for histology⁸: the morphology indeed is preserved; the IHC and the special stainings are comparable to those cases fixed in formalin.

Greenfix Plus is a more ethandial and ethyl alcohol concentrated solution that allows to fix whole organs and samples with large dimensions.

2.0 Bibliography

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3.0 GENERAL GUIDE LINES

This manual shows the guide lines concerning the right use of **GreenFix** and **GreenFix Plus** during first fixation and following samples cutting steps.

GreenFix is a completely formaldehyde free fixative, but with an aldehydic fixation process because ethandial based.

GreenFix is a new fixing mixture with preserving principles for the morphology and tissue antigenicity.

GreenFix use is the same as formalin; it operates at room temperature and it is compatible with all histoprocessors available on the market.

Diapath has created 2 formulations: **Greenfix** and **Greenfix Plus**:

- **GreenFix** is used to fix histological samples up to max thickness of 0.5-0.8 cm;
- **GreenFix Plus** is used to fix larger histological samples, organ parts or whole organs.

We suggest to use a volumetric relationship between sample and fixative as 1:20 at least and to follow the good practices concerning sample opening and sectioning to allow a right fixation.

We suggest to avoid a heat fixation.

The tissues fixed in **GreenFix** and **GreenFix Plus** lose the first staining but do not take up the uniform greyish characteristic stain given by the formalin fixation; for this reason they preserve an appearance similar to the fresh tissue.

The tissues fixed in **GreenFix** and **GreenFix Plus** have a stiff consistency that allows their cut and sampling. It is also possible to notice that the samples are less stiff than usual compactness of tissues fixed in formalin.

Once finished sample fixation process and started its cutting process, it is possible to keep the embedding cassettes with the sample into **GreenFix** up to begin the histoprocessing.

Inside the histoprocessors **GreenFix** can be used as first step to allow samples delayed start, keeping the samples in fixative and assuring their whole fixation.

The product has been tested in different Pathological Anatomy Institutes.

During the tests different kind of tissues were fixed in **GreenFix** and **GreenFix Plus**, subsequently exposed to hematoxylin and eosin staining, immunohistochemical surveys and histochemical special staining.

Tested tissues are:

Breast, pancreas, spleen, salivary glands, smooth and striated muscular tissue, thymus, brain, kidney, adrenal gland, lymph gland, liver, large intestine, lung, uterus, gall bladder, stomach, hypophysis.

Different dimensions samples have been fixed from few mm of thickness (small biopsies) to whole organs (liver, pancreas); samples with small dimensions (or fresh cut samples inserted into cassettes) have been fixed in **GreenFix**; instead organ parts or whole organs in **GreenFix Plus**.

The samples have been placed into the fixative from 12 hours to 5-6 days.

Following the cutting the samples have been overnight processed using ethyl alcohol and xylol, xylol substitutes and Ottix (substitute of alcoholic scale and xylol).

The obtained results with hematoxylin eosin stainings, immunohistochemical surveys and special histochemical stainings are optimal and comparable with control samples fixed in formalin.

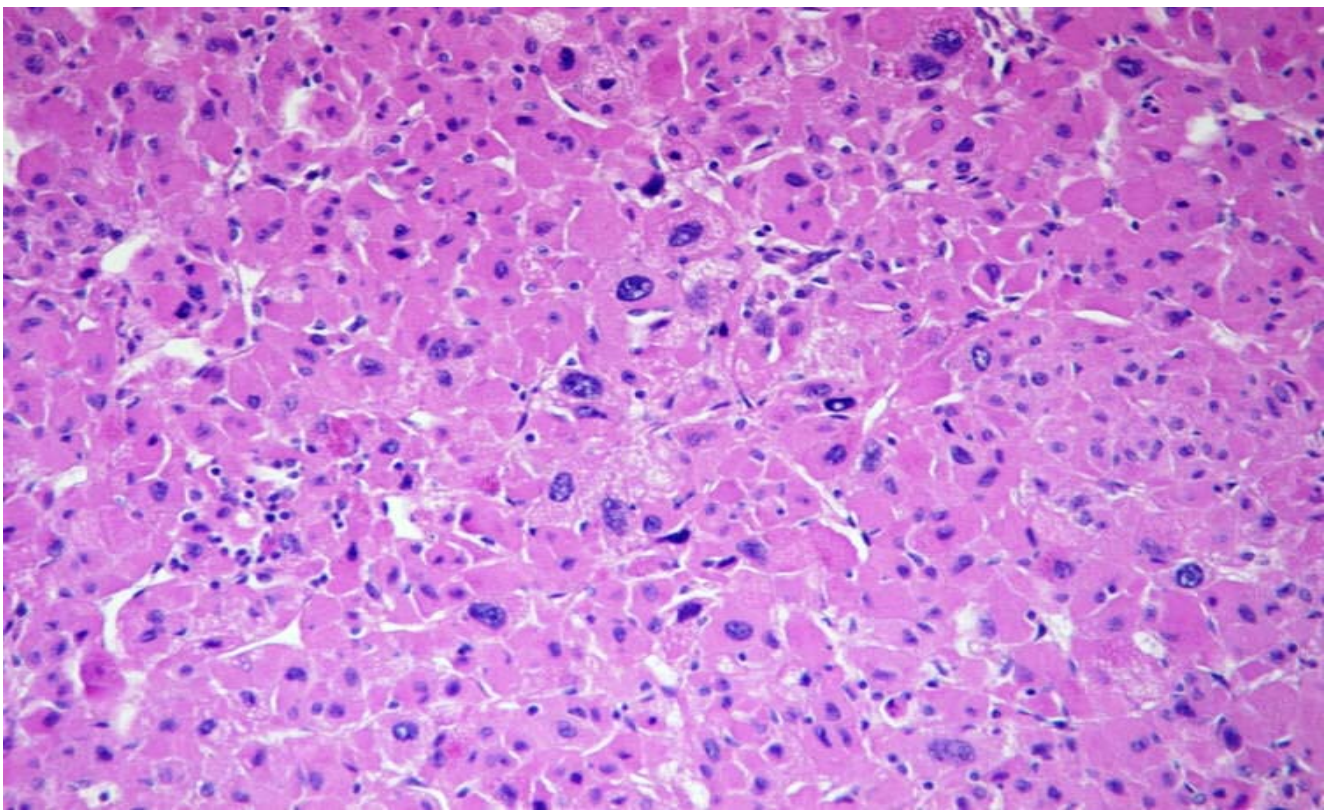
GreenFix is compatible with all histoprocessors and reagents used in Pathological Anatomy on the market.

Diapath puts its experience at disposal to use **GreenFix**, providing some guide lines concerning the histoprocessing, hematoxylin and eosin staining, special histochemical staining and immunohistochemical procedures.

4.0 HISTOPROCESSING

4.1 HISTOPROCESSING PROTOCOL EXAMPLE WITH ALCOHOLS AND XYLOL:

REAGENT	TIME	TEMPERATURE	PRESSURE/VACUUM
Alcohol 70	Delayed Start	37°C	P/V
Alcohol 80	45'	37°C	P/V
Alcohol 95	45'	37°C	P/V
Alcohol 95	1h	37°C	P/V
Absolute alcohol	45'	37°C	P/V
Absolute alcohol	1h	37°C	P/V
Xylene	45'	37°C	P/V
Xylene	1h	37°C	P/V
Paraffin	30'	58°C	P/V
Paraffin	45'	58°C	P/V
Paraffin	45'	58°C	P/V
Paraffin	1h	58°C	P/V

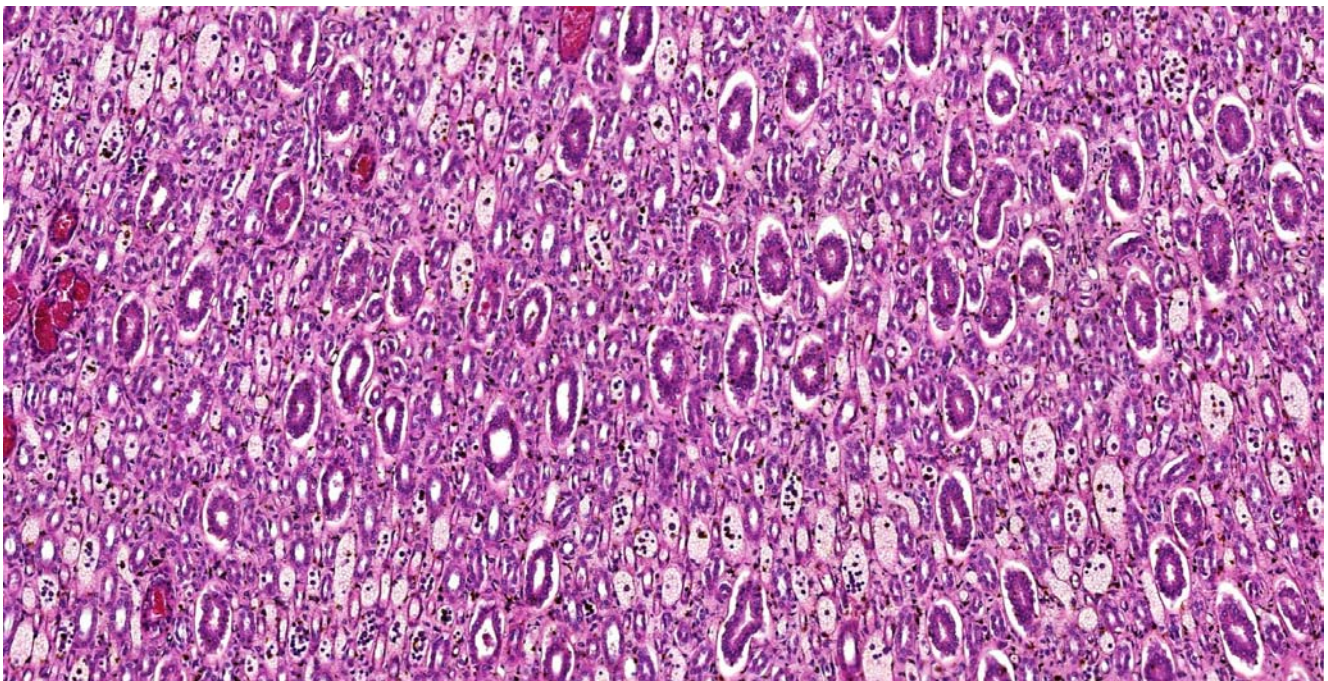


Adrenal Gland 20X– Hematoxylin Eosin

4.2 HISTOPROCESSING PROTOCOL EXAMPLE IN OTTIX:

REAGENT	TIME	TEMPERATURE	PRESSURE/VACUUM	MIX
GreenFix	10'	RT	/	3
Ottix Shaper	30'	RT	/	3
Ottix Plus	1h	37 °C	P/V	3
Ottix Plus	1h	37 °C	P/V	3
Ottix Plus	2h	37 °C	P/V	3
Ottix Plus	2h	37 °C	P/V	3
Paraffin	1h	60 °C	P/V	3
Paraffin	1h 30'	60 °C	P/V	3
Paraffin	1h 30'	60 °C	P/V	3

RT= Room Temperature

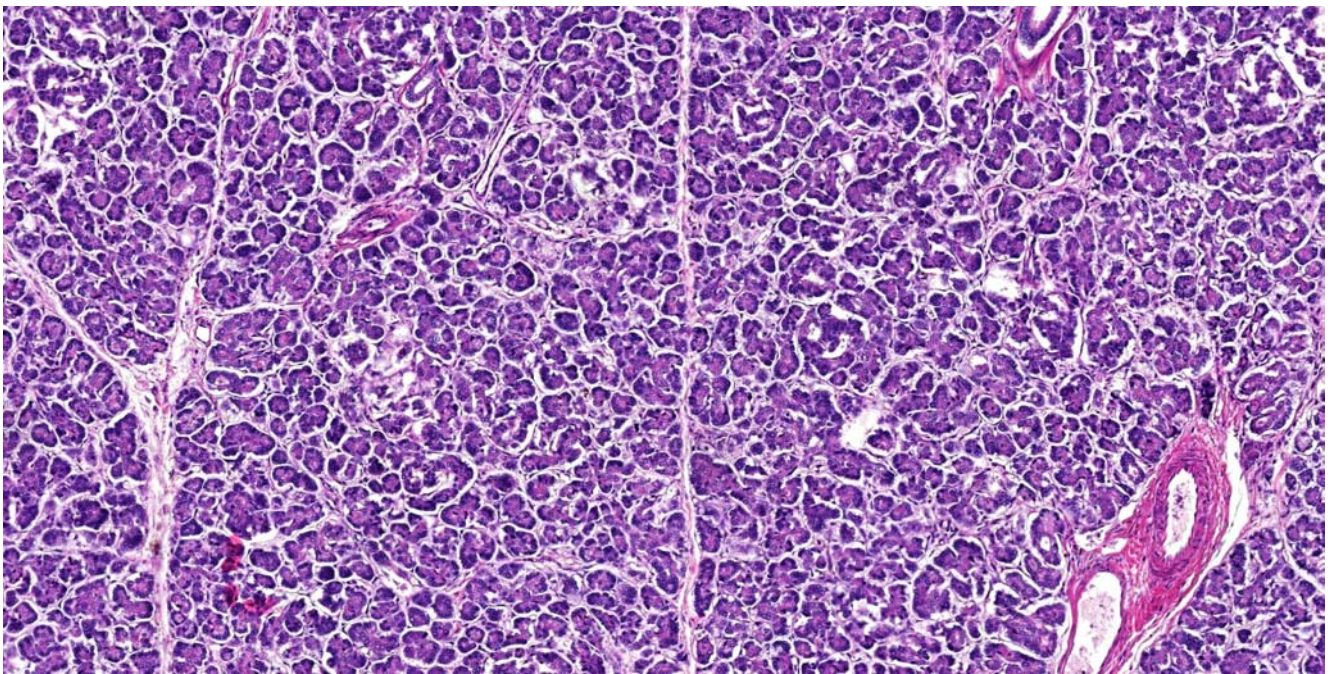


Kidney 20X – Hematoxylin Eosin

5.0 HEMATOXYLIN EOSIN STAINING

5.1 DEPARAFFINATION PROTOCOL EXAMPLE AND STAINING WITH ALCOHOLS AND XYLOL:

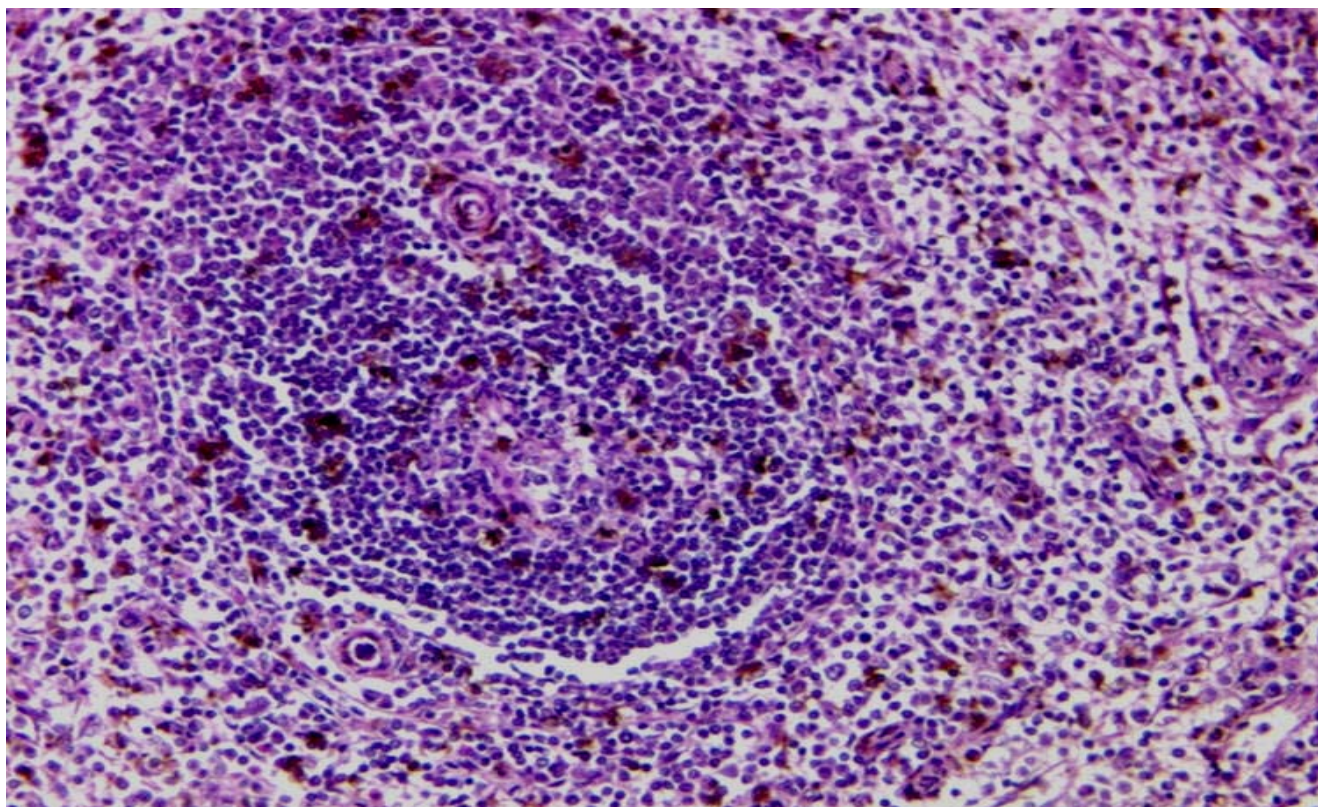
REAGENT	TIME
Xylol or substitutes	5'
Xylol or substitutes	5'
Absolute alcohol (histoalcol 99)	5'
Absolute alcohol (histoalcol 99)	5'
Alcohol 95 (histoalcol 95)	5'
Running water	5'
Hematoxylin	5'
Running water	10'
Alcoholic Eosin	3'
Alcohol 95 (histoalcol 95)	10 dips (30")
Alcohol 99 (histoalcol 99)	5'
Alcohol 99(histoalcol 99)	5'
Xylol or substitutes	5'



Pancreas 20X – Hematoxylin Eosin

5.2 DEPARAFFINATION PROTOCOL EXAMPLE AND STAININGS IN OTTIX:

REAGENT	TIME
Ottix Plus	7'
Ottix Plus	7'
Ottix Shaper	3'
Running water	5'
Hematoxylin	5'
Running water	5'
Alcoholic Eosin	30"-1'
Ottix Shaper	30"
Ottix Shaper	30"
Ottix Plus	5'
Ottix Plus	5'



Spleen 20X– Hematoxylin Eosin

6.0 IMMUNOHISTOCHEMISTRY

Revelation kits used for immunohistochemical protocols are:

- Polymeric revelation kit (DAKO ENVISION)
- Polymeric revelation kit (LABVISION Thermo Scientific)
- Multimeric revelation kit (ultraVIEW Dab Detection kit VENTANA)

For the immunohistochemical marking the diaminobenzidine (DAB) has been used.

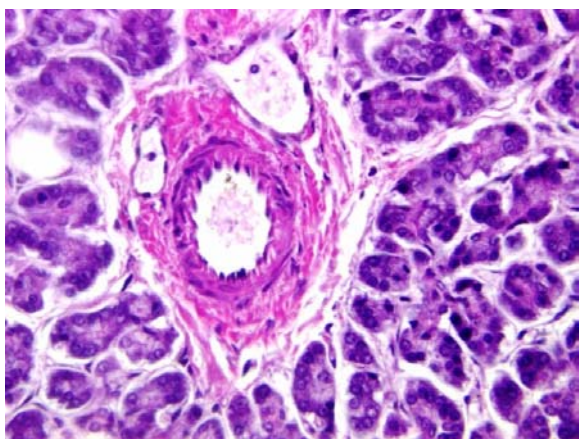
Diapath suggests to not use high concentration protease enzyme as enzymatic pre-treat for a time more than 3/5 minutes because it could digest the tissue fixed in **GreenFix** and **GreenFix Plus**. Alternatively we suggest to replace the enzymatic pre-treat with thermic one.

Histological samples preservation for time longer than normal in **GreenFix Plus** provokes overfixation artefacts with changes to immunohistochemical answer and antigen retrieval protocols (conditions similar to formalin overfixation).

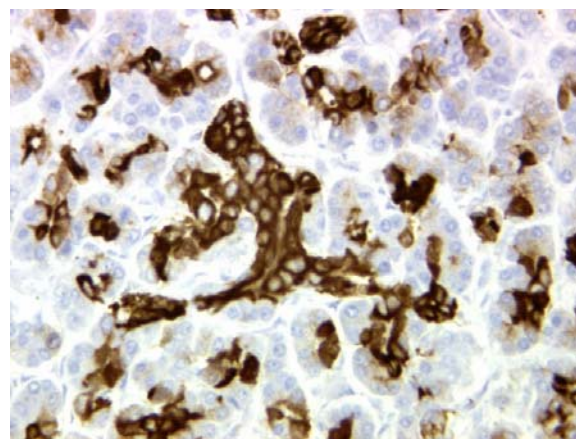
6.1 PANCREAS

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
Keratin Pool	AE1/AE3	1:75	Citrate	6	20'	98	30' RT
Chromogranin	LK2H10+PHE5	1:1000	Citrate	6	40'	98	30' RT
Keratin 7	OV-TL 12/30	1:40	Citrate	6	10'	98	60' RT
Keratin 7	OV-TL 12/30	1:100	Citrate	6	30'	98	30' RT
Keratin 20	Ks 20.8	1:50	Citrate	6	10'	98	30' RT
Synaptophysin	polyclonal	1:60	Citrate	6	40'	98	60' RT
NSE	E27	1:100	Citrate	6	40'	98	60' RT
Keratin 19	RCK 108	1:50	Citrate	6	10'	98	60' RT

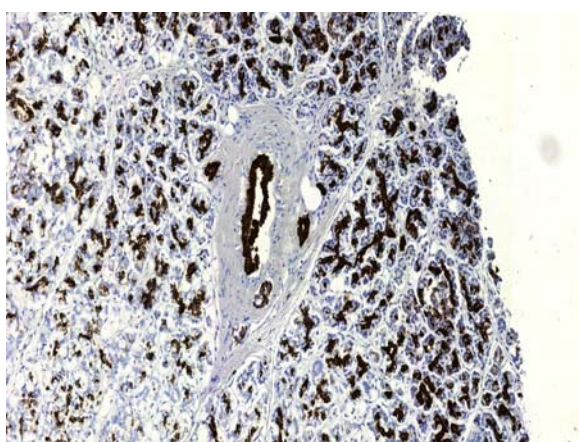
RT= Room Temperature



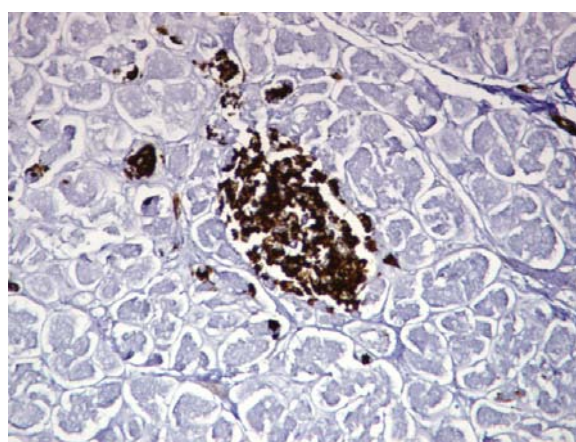
Pancreas 40X – Hematoxylin Eosin



Pancreas 40X – Keratin 7



Pancreas 20X -Chromogranin

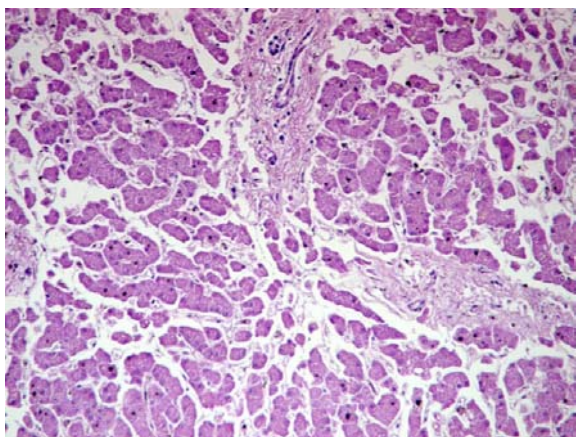


Pancreas 10X – Keratin 19

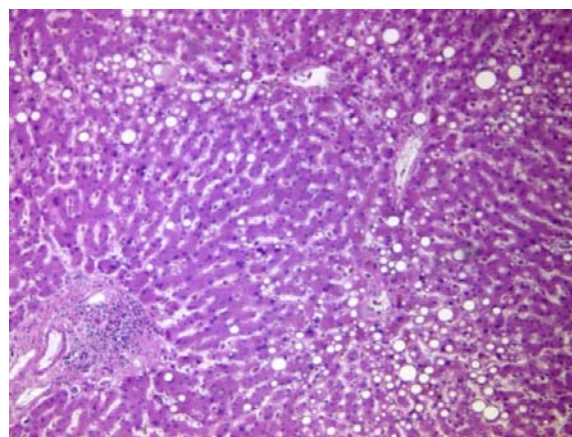
6.2 LIVER

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
Keratin Pool	AE1/AE3	1:50	Citrate	6	30'	98	30' RT
Keratin Pool	AE1/AE3	1:75	Citrate	6	20'	98	30' RT
CD 31	PECAM-1	1:50	EDTA	8	10'	98	60' RT
Epar-1	OCHIE5	1:25	EDTA	9	25'	98	30' RT

RT= Room Temperature



Liver 20X – Hematoxylin Eosin

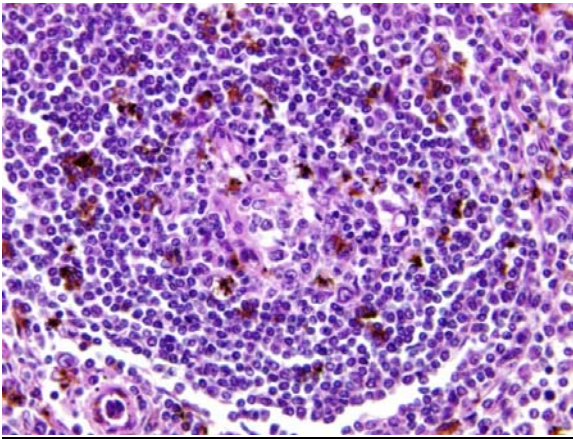


Liver 10X - Hematoxylin Eosin

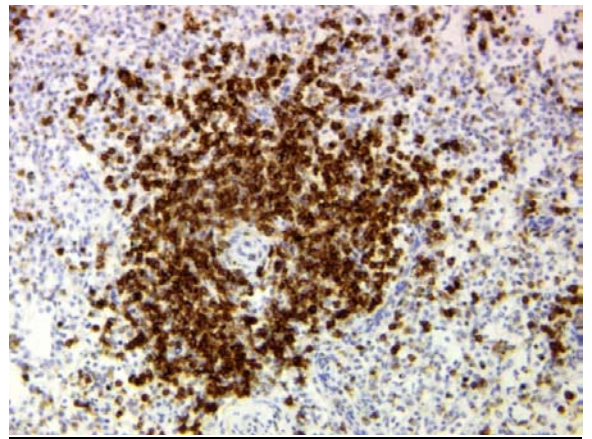
6.3 SPLEEN

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
CD 20	L26	1:350	Citrate	6	15'	98	40' RT
CD 79a	HM47/A9	1:500	EDTA	8	30'	98	40' RT
CD 34	QBend/10	1:500	EDTA	8	30'	98	40' RT
CD 31	PECAM-1	1:50	EDTA	8	10	98	60' RT

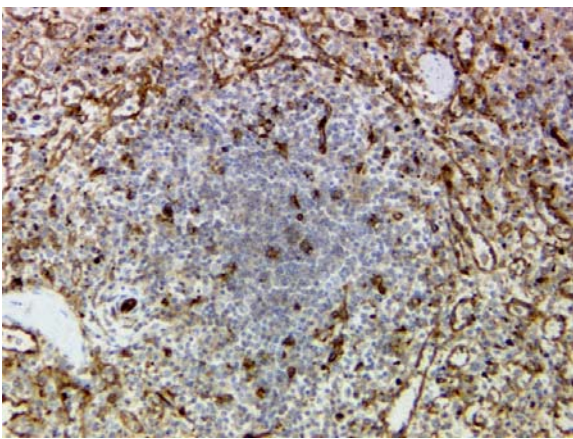
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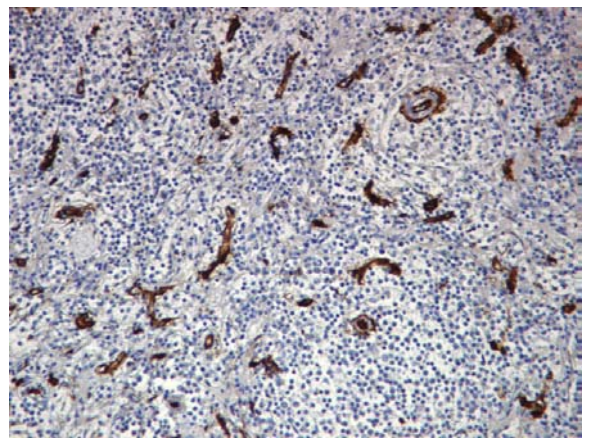
Spleen 40 X - Hematoxylin Eosin



Spleen 20X - CD 20



Spleen 20X - CD 31

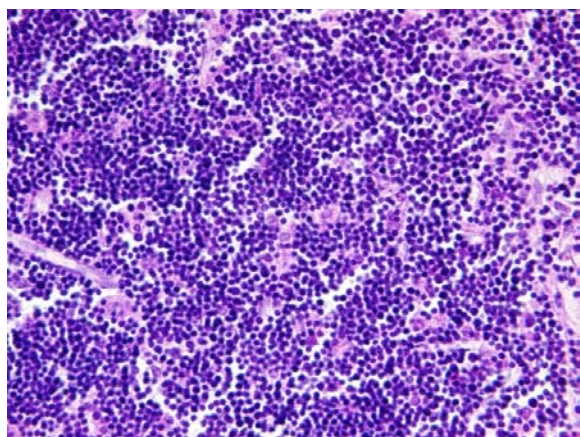


Spleen 20X - CD 34

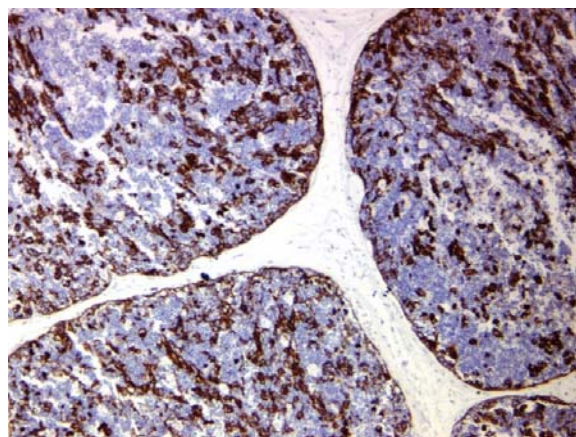
6.4 THYMUS

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
CD 30	VER-H2	1:40	EDTA	8	10'	98	60' RT
CD 79a	HM47/A9	1:500	EDTA	8	30'	98	40' RT
CD 8	C8/144B	1:25	Citrate	6	40'	98	60' RT
CD 3	PC3/188A	1:100	EDTA	9	20'	98	30' RT
Keratin Pool	AE1/AE3	1:50	Citrate	6	30'	98	30' RT
Ki 67	K2	Prediluted	CC1 standard				32' a 37°C

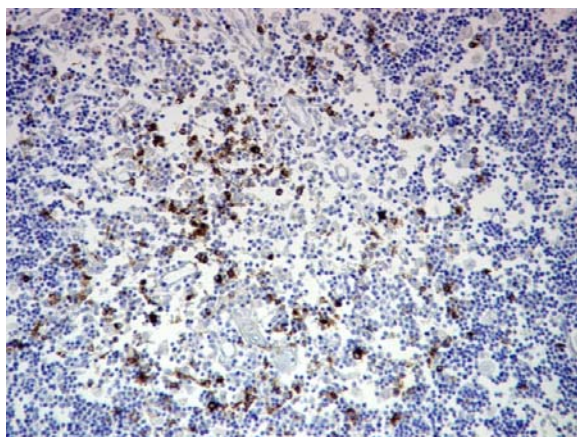
RT= Room Temperature



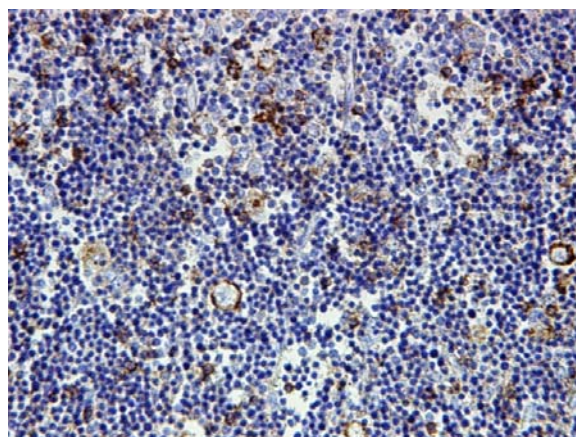
Thymus 20X - Hematoxylin Eosin



Thymus 10X – Keratin Pool



Thymus 20X - CD79a

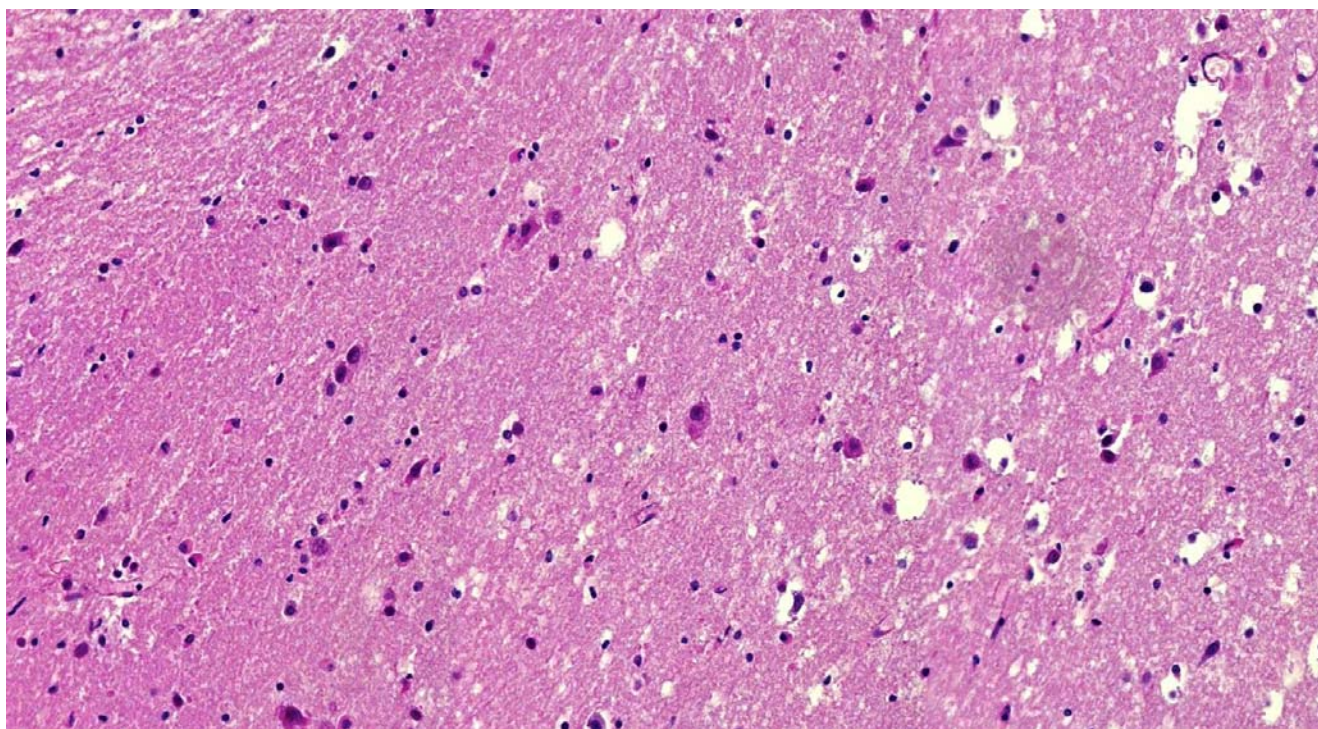


Thymus 40X - CD8

6.5 BRAIN

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
GFAP	6F2	1:50	EDTA	8	10'	98	40' RT
GFAP	6F2	1:100	NOT PRE-TREATED				30' RT

RT= Room Temperature

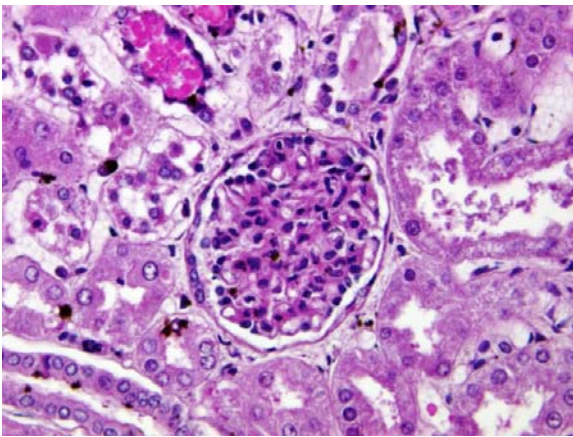


Brain 20X - Hematoxylin Eosin

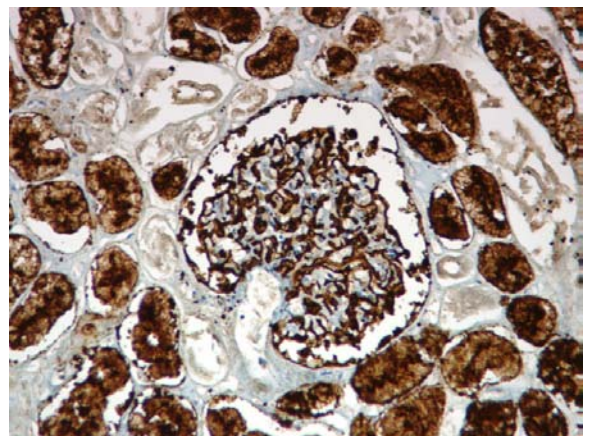
6.6 KIDNEY

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
Collagen IV	CIV 22	1:50	EDTA	8	10'	98	40' RT
EMA	E29	1:50	NOT PRE-TREATED				30' RT
CD 34	QBend/10	1:500	EDTA	8	30'	98	40' RT
CD 68	KP1	1:500	EDTA	8	10'	98	60' RT
CD 31	PECAM-1	1:50	EDTA	8	10'	98	60' RT
CD 10	56C6	Prediluted	CC1 standard				72' RT
CD 10	56C6	1:30	EDTA	9	25'	98	30' RT

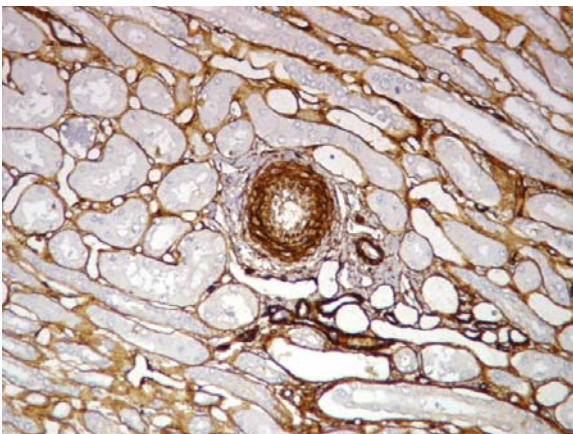
RT= Room Temperature



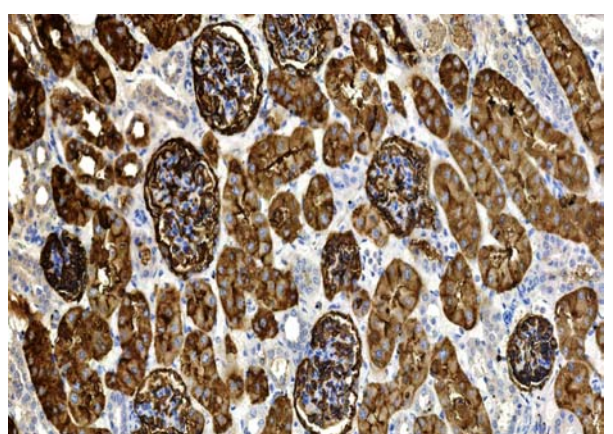
Kidney 40X - Hematoxylin Eosin



Kidney 40X - CD 10



Kidney 20X - Collagen IV

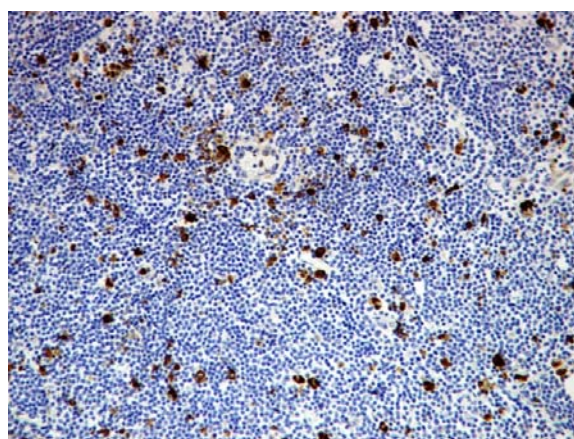


Kidney20X - CD 10

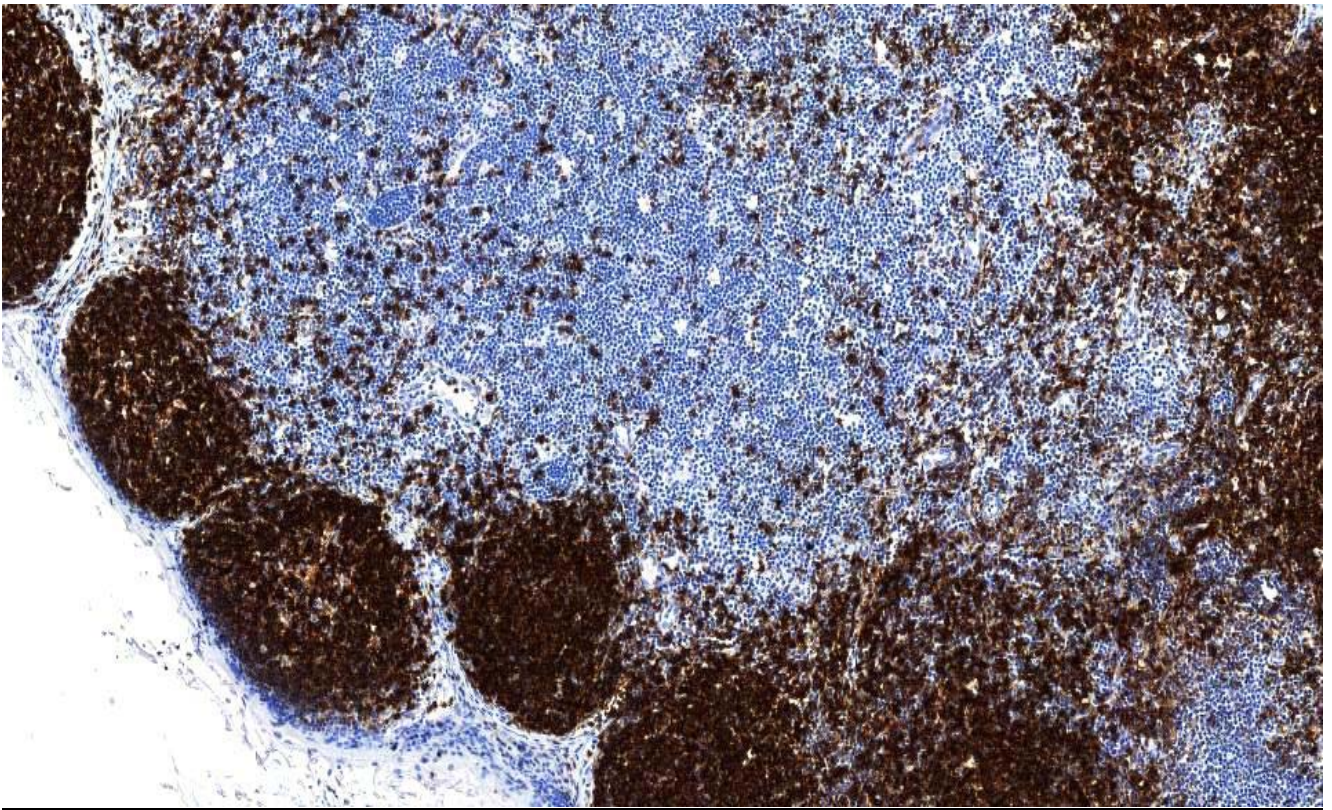
6.7 LYMPH NODE

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			Buffer	pH	t	T°C	
CD 79a	HM47/A9	1:500	EDTA	8	30'	98	40' RT
CD 20	L26	1:350	Citrate	6	15'	98	40' RT
CD 20	L26	1:200	Citrate	6	30'	98	15' RT
CD 3	Polyclonal	1:150	Citrate	6	45'	98	60' RT
CD 3	PC3/188A	1:100	EDTA	9	20'	98	30' RT
CD 43	DF-T1	1:50	NOT PRE-TREATED				40' RT
CD 45 LCA	PD7/26/16 + 2B11	1:100	NOT PRE-TREATED				60' RT
Collagen IV	CIV 22	1:50	EDTA	8	10'	98	40' RT
CD 68	KP1	1:200	Citrate	6	10'	98	15' RT
Kappa chain	Polyclonal	1:25000	Triypsin	7,8	30'	37	15' RT
Lambda chain	FITC	1:50000	Triypsin	7,8	30'	37	15' RT
Ki 67	K2	Prediluted	CC1 standard				32' to 37°C

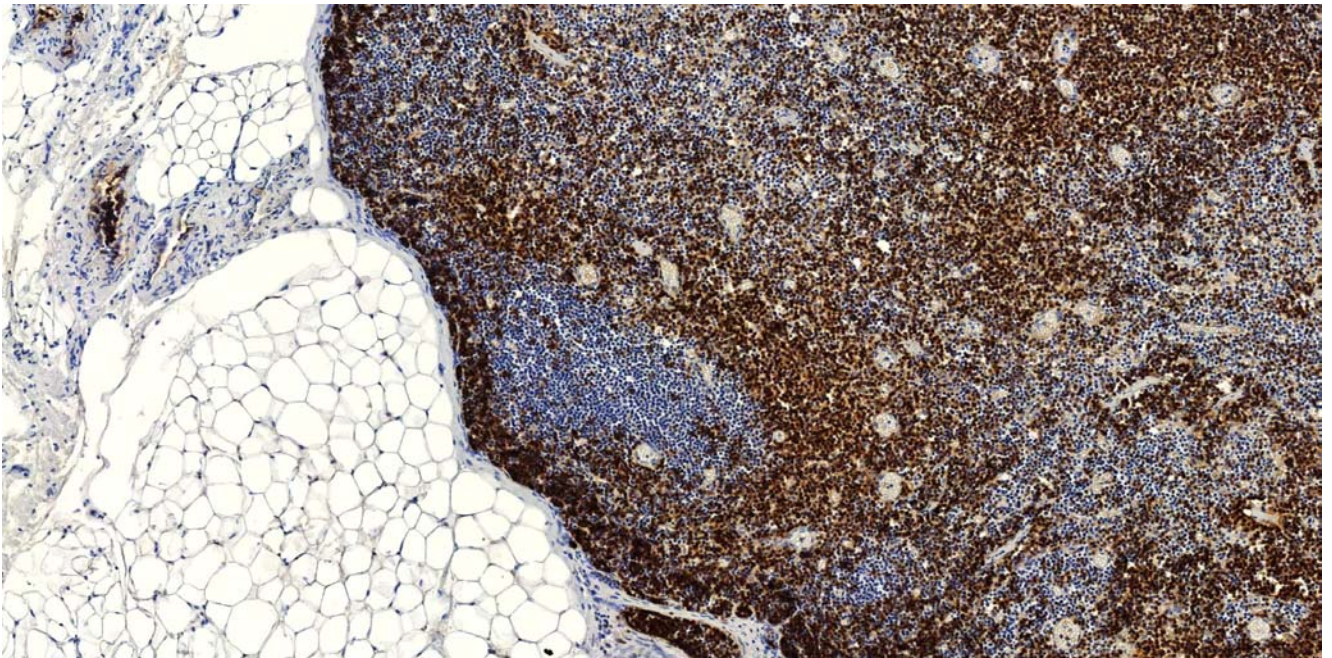
RT= Room Temperature



Lymph Node 20X - CD 68



Lymph Node 10X - CD20



Lymph Node 10X - CD3

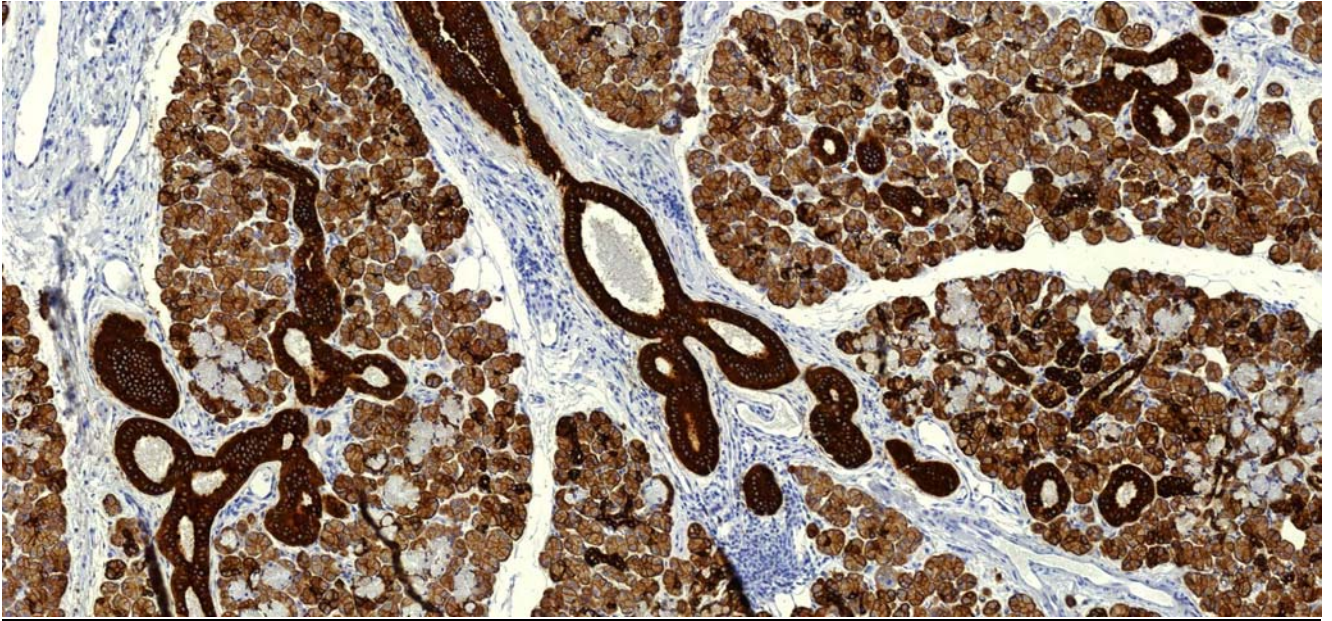
6.8 SALIVARY GLANDS

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			Buffer	pH	t	T°C	
Keratin Pool	AE1/AE3	1:75	Citrate	6	20'	98	30' RT
Keratin Pool	AE1/AE3	1:50	Citrate	6	30'	98	30' RT
S-100	Polyclonal	Prediluted	NOT PRE-TREATED				15' RT
S-100	Polyclonal	1:200	EDTA	8	5'	98	30' RT
Keratin 8/18	5D3	1:50	Citrate	6	40'	98	40' RT
Keratin 8/18	CAM 5.2	Prediluted	NOT PRE-TREATED				15' RT

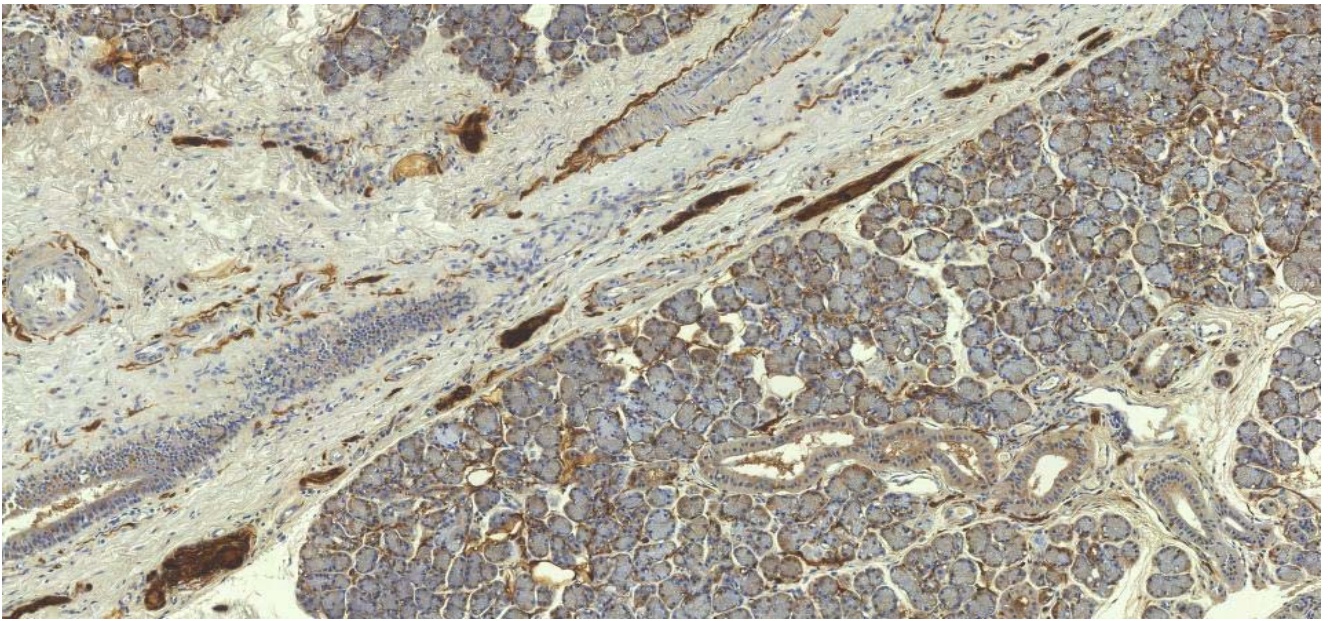
RT= Room Temperature



Salivary glands 20X – Keratin 8/18



Salivary glands 10X – Keratin Pool

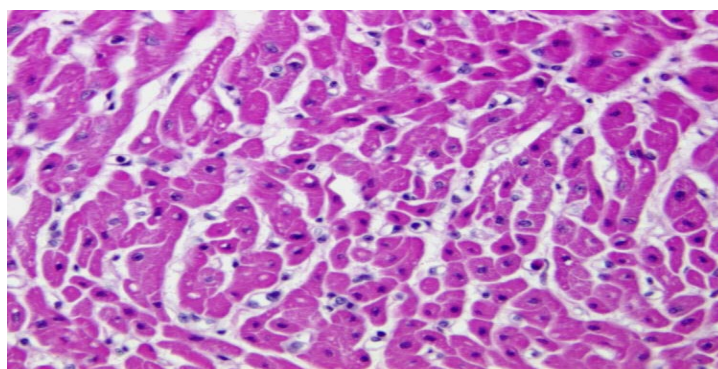


Salivary glands 10X - S-100

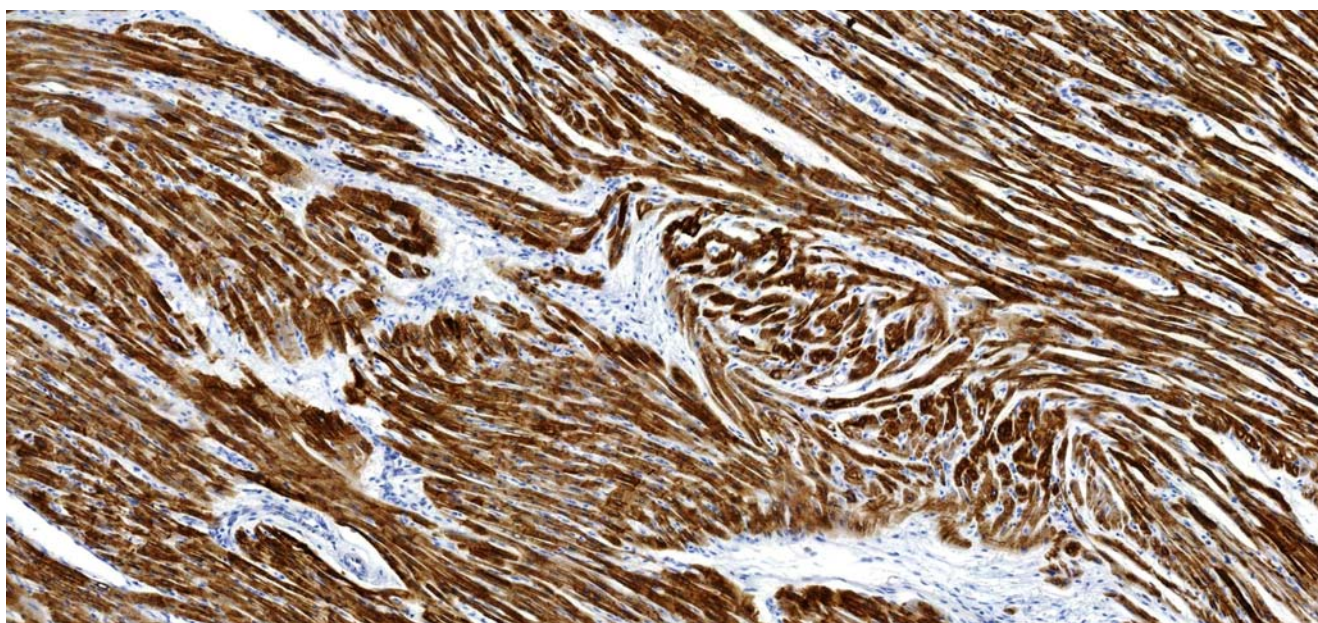
6.9 MYOCARDIUM

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
Actin specific muscle	HHF35	1:50	Citrate	6	10'	98	60' RT
Actin smooth muscle	1A4	1:2000	Citrate	6	10'	98	40' RT
Desmin	D33	1:75	Citrate	6	30'	98	15' RT

RT= Room Temperature



Myocardium 40X – Hematoxylin Eosin

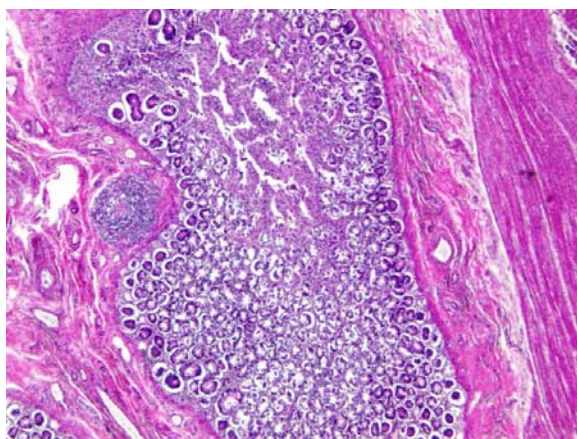


Myocardium 10X – DESMIN

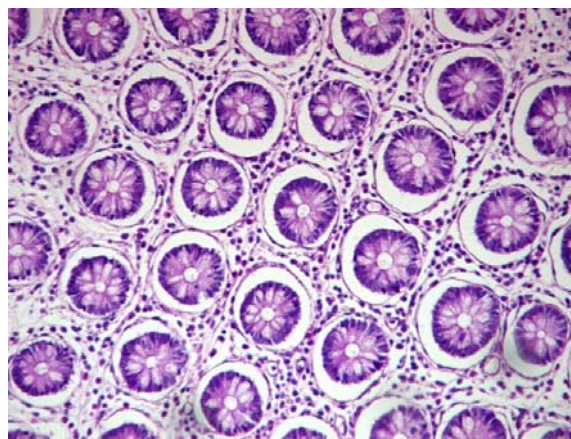
6.10 INTESTINE

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
P 63	63P02 <i>same as</i> Y4A3	1:100	EDTA	8	30'	98	30' RT
Vimentin	V9	1:1000	Citrate	6	30'	98	30' RT
Keratin Pool	AE1/AE3	1:75	Citrate	6	20'	98	30' RT
P 53	DO-7	1:200	Citrate	6	30'	98	30' RT
S-100	Polyclonal	1:200	EDTA	8	10'	98	30' RT
Keratin 20	Ks 20.8	1:500	Triypsin 0,1%	7,8	30'	30	30' RT
Ki 67	30.9	Prediluted	CC1 reduced				32' to 37°C
Ki 67	30.9	Prediluted	CC1 standard				32' to 37°C
Ki 67	30.9	Prediluted	CC1 extended				32' to 37°C
Ki 67	30.9	Prediluted	CC1 reduced				60' RT
Ki 67	30.9	Prediluted	CC1 reduced				32' to 42°C
Ki 67	30.9	Prediluted	CC1 reduced				16' to 37°C

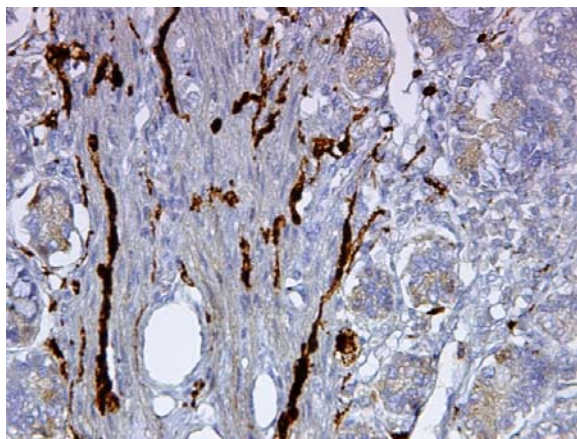
RT= Room Temperature



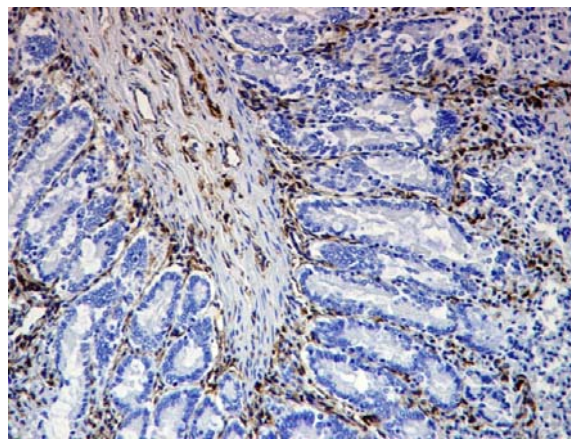
Intestine 5X – Hematoxylin Eosin



Intestine 20X - Hematoxylin Eosin



Intestine 40X - S-100

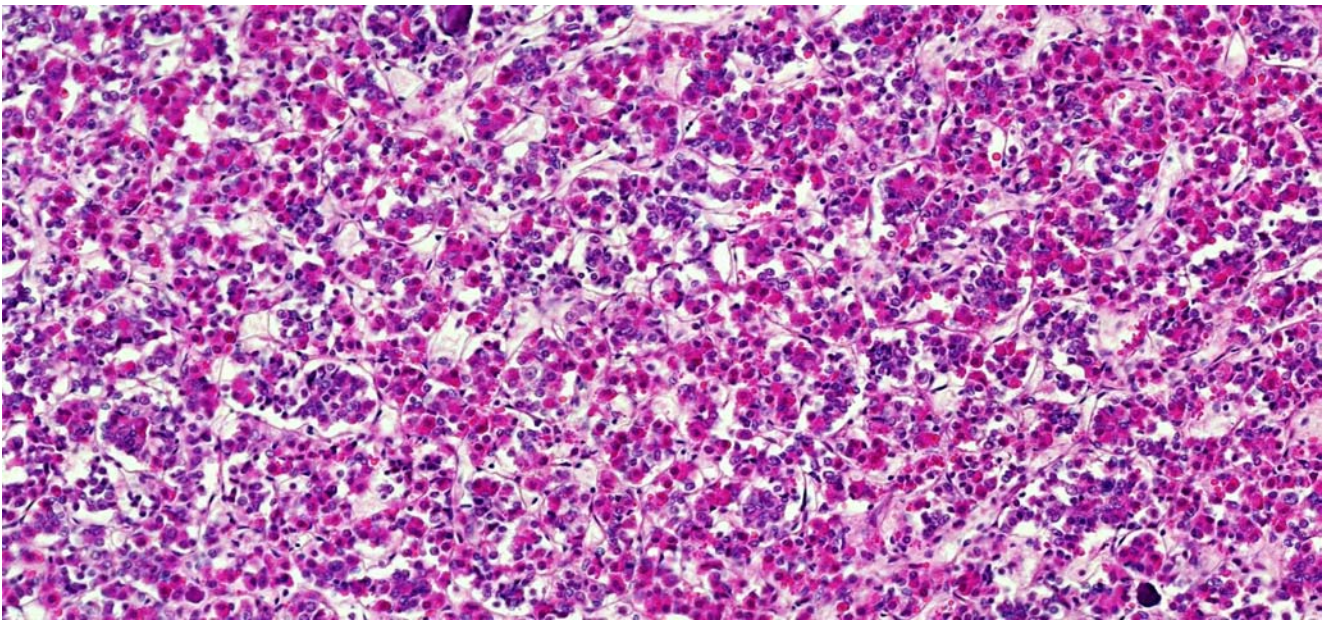


Intestine 10X - Vimentin

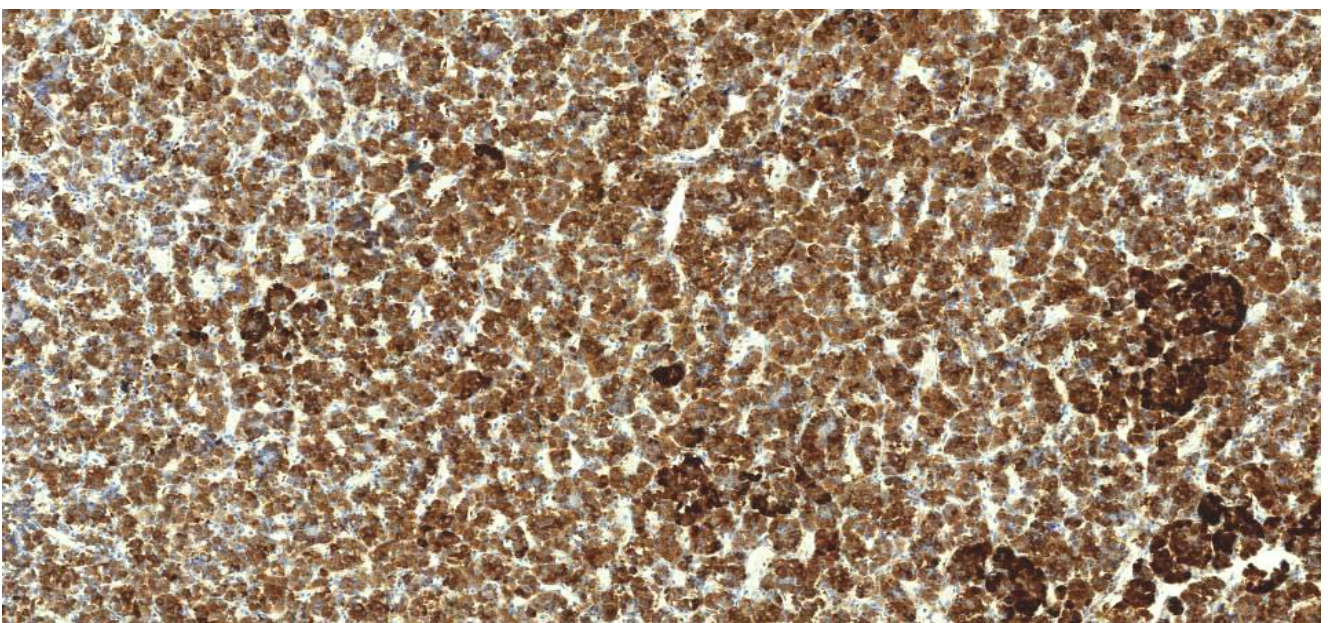
6.11 HYPOPHYSIS

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
Synaptophysin	SY38	1:30	Citrate	6	30'	98	15' RT
Chromogranin	DAK-A3	1:150	Citrate	6	30'	98	15' RT

RT= Room Temperature



Hypophysis 40X - Hematoxylin Eosin

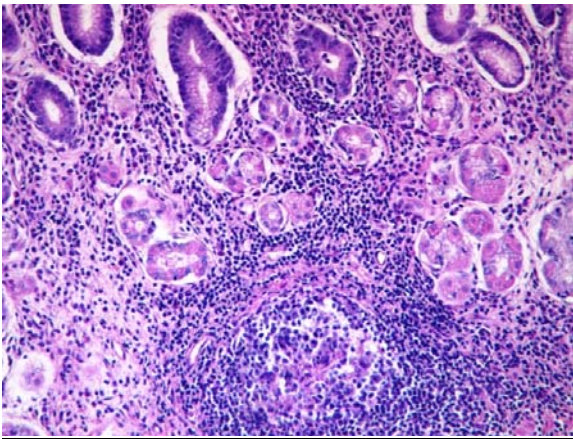


Hypophysis 20X – Synaptophysin

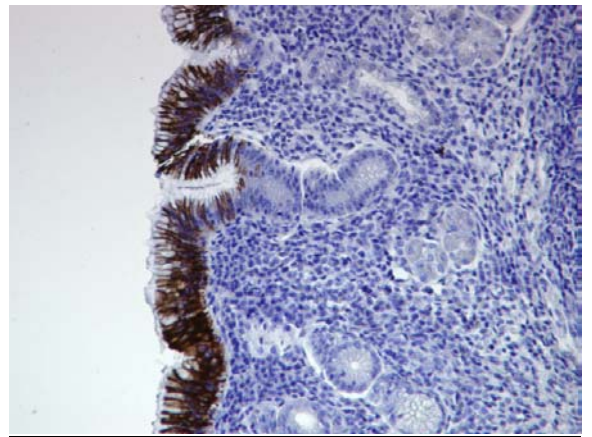
6.12 STOMACH

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
Keratin 20	Ks 20.8	1:50	EDTA	8	10'	98	30' RT
NSE	E27	1:100	Citrate	6	40'	98	60' RT

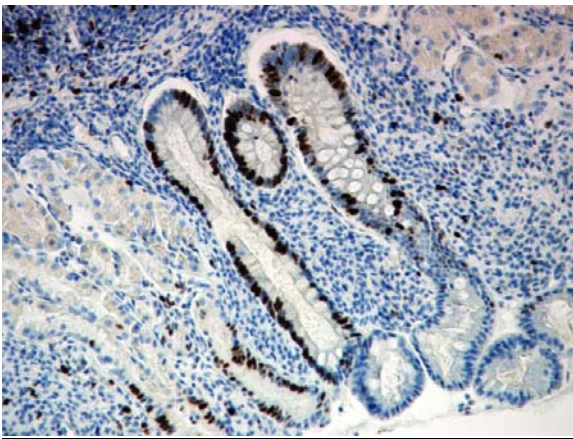
RT= Room Temperature



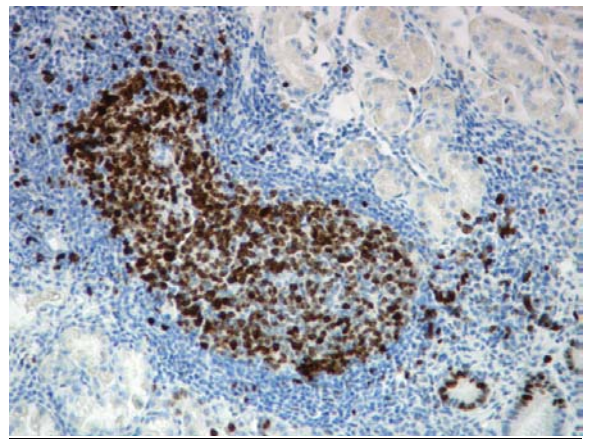
Stomach 20X – Hematoxylin Eosin



Stomach 20X – Keratin 20



Stomach 20X - Ki67

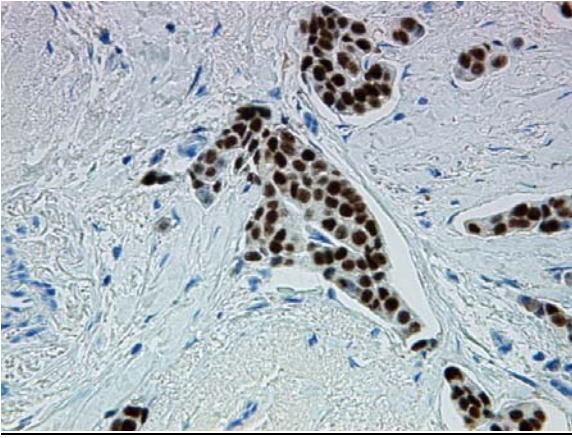


Stomach 20X - Ki67

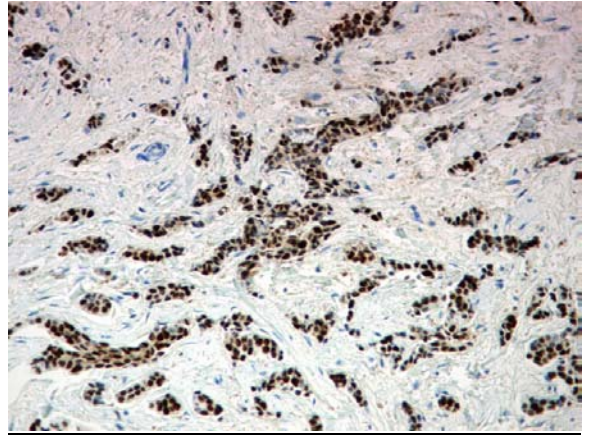
6.13 BREAST

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
Estrogen Receptor	SP1	1:40	EDTA	8	60'	98	40' RT
Estrogen Receptor	SP1	Prediluted	CC1 reduced				30' to 37°C
Estrogen Receptor	SP1	Prediluted	CC1 reduced				12' to 37°C
Estrogen Receptor	SP1	1:150	EDTA	8	45'	98	45' RT
Estrogen Receptor	1D5+6F11	1:150	EDTA	8	2'	100	Overnight 4°C
Progesteron Receptor	PgR 636	1:40	EDTA	8	90'	98	40' RT
Progesteron Receptor	1E2	Prediluted	CC1 reduced				30' to 37°C
Progesteron Receptor	1E2	Prediluted	CC1 reduced				12' to 37°C
Progesteron Receptor	1E2	Prediluted	CC1 reduced				20' to 37°C
Progesteron Receptor	Ab8	Prediluted	EDTA	8	45'	98	45' RT
Progesteron Receptor	1A6	1:50	EDTA	8	2'	100	Overnight 4°C
Ki 67	30.9	Prediluted	CC1 reduced				32' to 37°C
Ki 67	Mib-1	1:25	EDTA	8	45'	98	45' RT
Ki 67	Mib-1	1:90	EDTA	8	2'	100	Overnight 4°C
P 53	DO-7	1:150	EDTA	8	2'	100	Overnight 4°C
Her-2	Polyclonal	1:25	Citrate	6	20'	98	30' RT

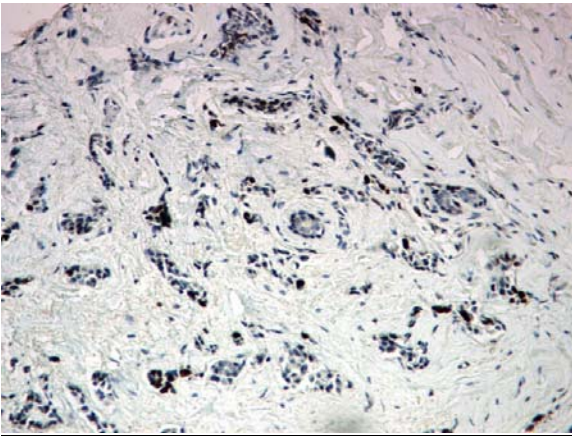
RT= Room Temperature



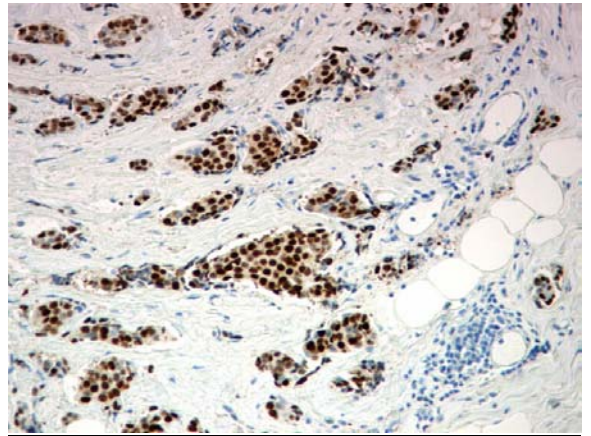
Breast 40X – ESTROGEN RECEPTOR



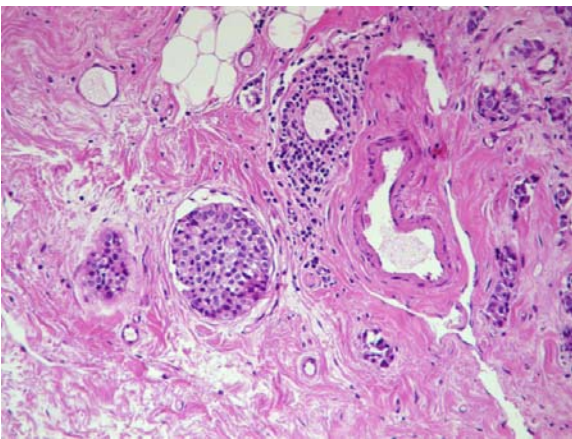
Breast 20X - ESTROGEN RECEPTOR



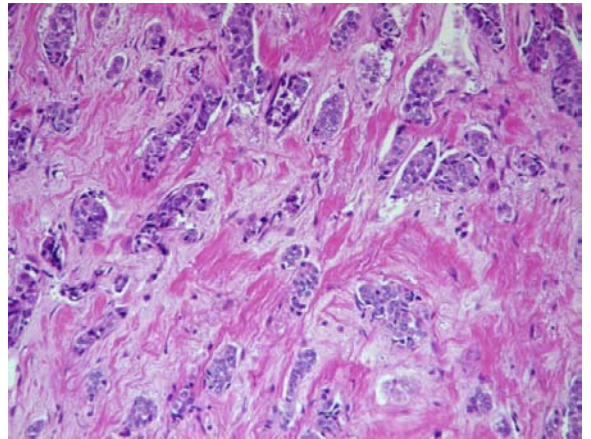
Breast 20X - Ki67



Breast 20X – PROGESTERON RECEPTOR



Breast 20X – Hematoxylin Eosin

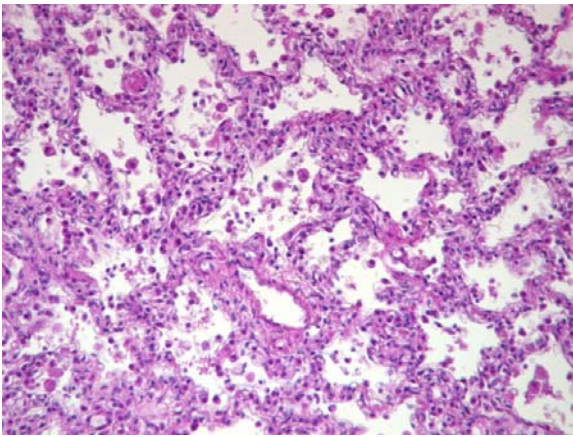


Breast 20X - Hematoxylin Eosin

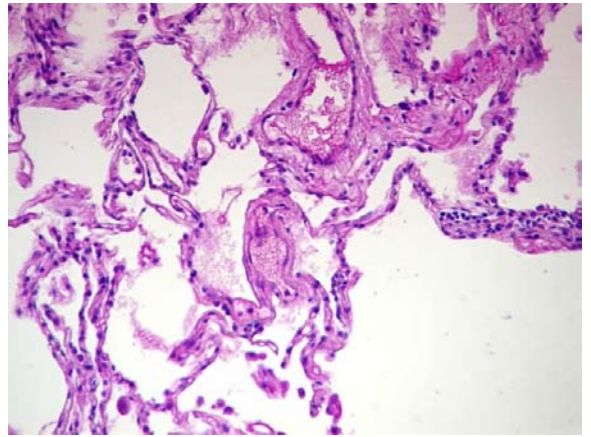
6.14 LUNG

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
TTF-1	8G7G3/1	1:500	EDTA	8	60'	98	60' RT

RT= Room Temperature



Lung 20X - Hematoxylin Eosin

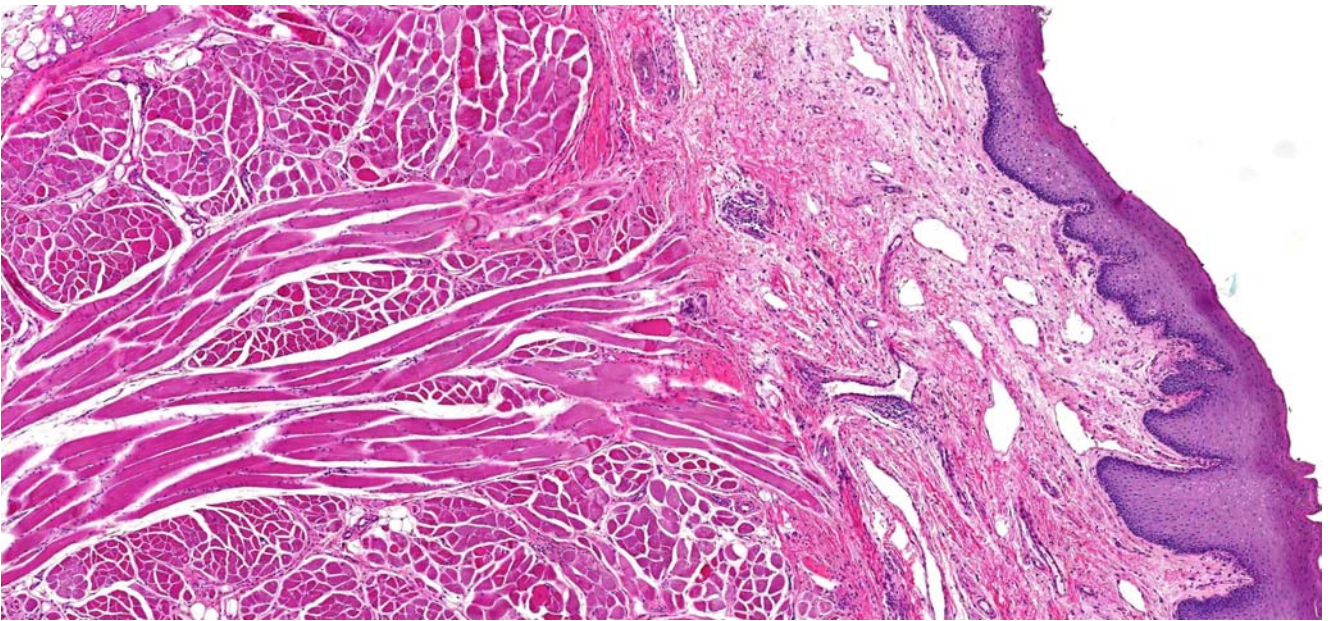


Lung 20X - Hematoxylin Eosin

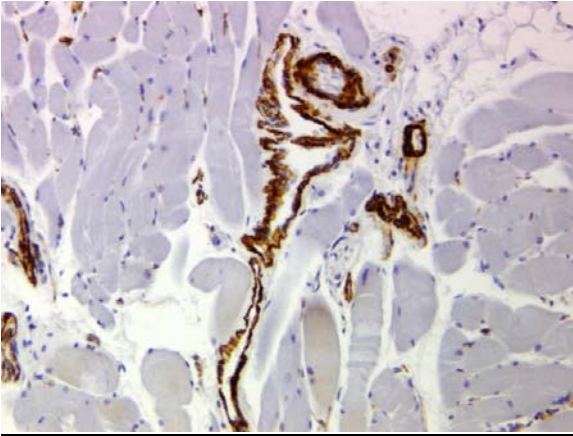
6.15 TONGUE

ANTIBODY	CLONE	DILUTION	Antigen retrieval				INCUBATION
			buffer	pH	t	T°C	
Actin smooth muscle	1A4	1:2000	Citrate	6	10'	98	40' RT
Keratin 14	LL022	1:50	Citrate	6	30'	98	60' RT
P 53	DO-7	1:200	Citrate	6	30'	98	40' RT

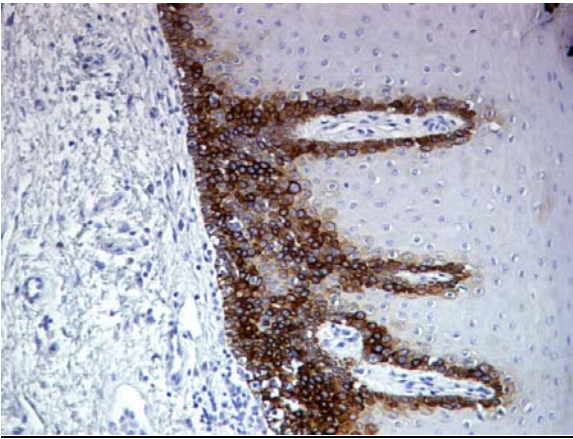
RT= Room Temperature



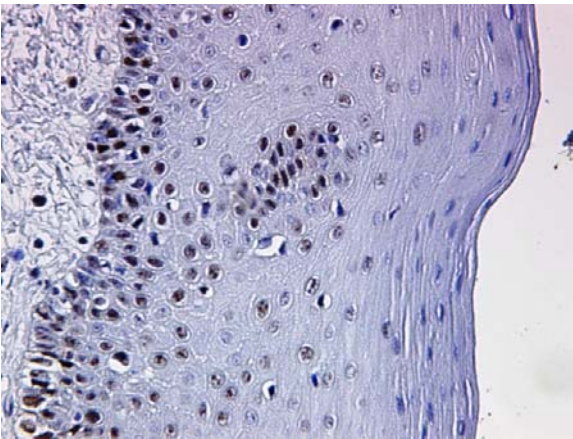
Tongue 5X - Hematoxylin Eosin



Tongue 20X - Actin Smooth Muscle



Tongue 20X - Keratin 14



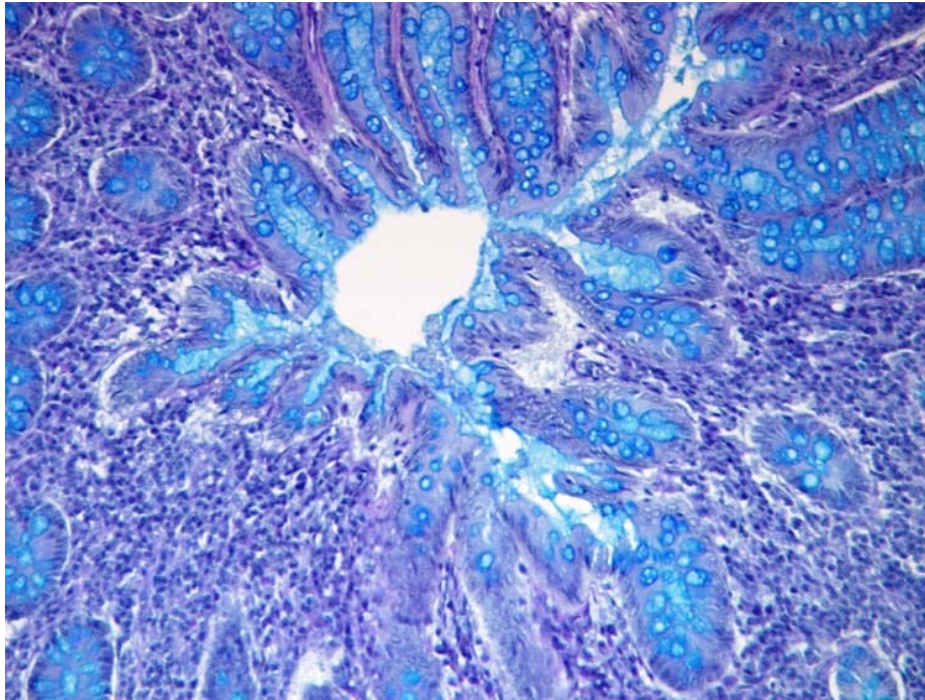
Tongue 40X - P53

7.0 SPECIAL STAININGS

Diapath suggests to use for the special stainings the same protocols for the samples fixed in formalin.

Here following some stainings examples on samples fixed in **GreenFix** and **GreenFix Plus**:

7.1 Alcian Blue pH 2,5- P.A.S. acc. Mowry



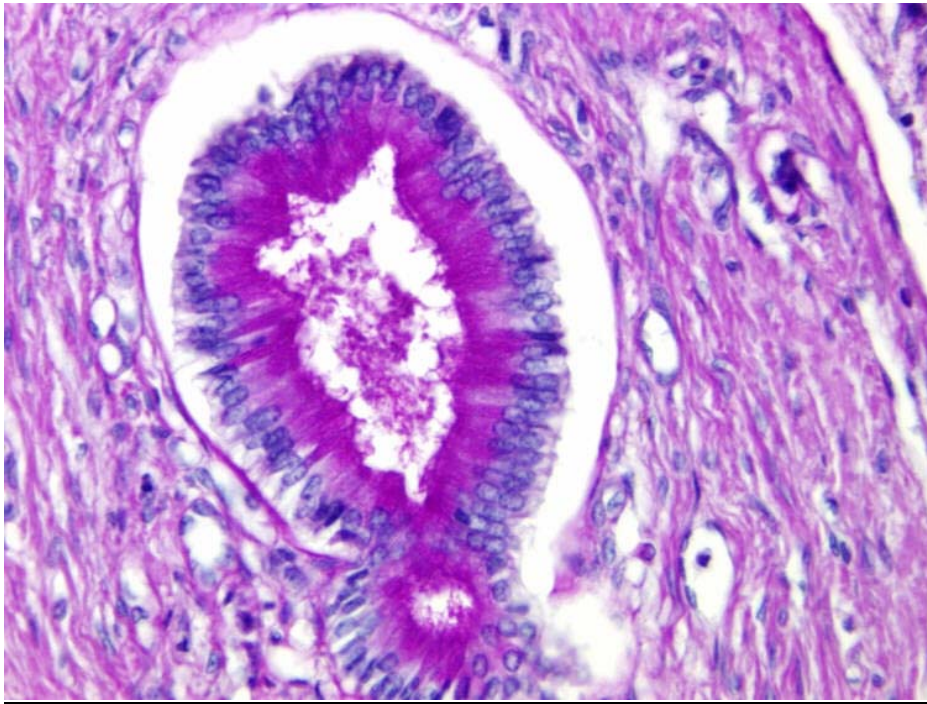
Intestine 20X- ALCIAN BLUE PAS

Mucins: blue-turquoise

Nucleus: blue

PAS positive substance: magenta

7.2 P.A.S.- Periodic Acid Schiff

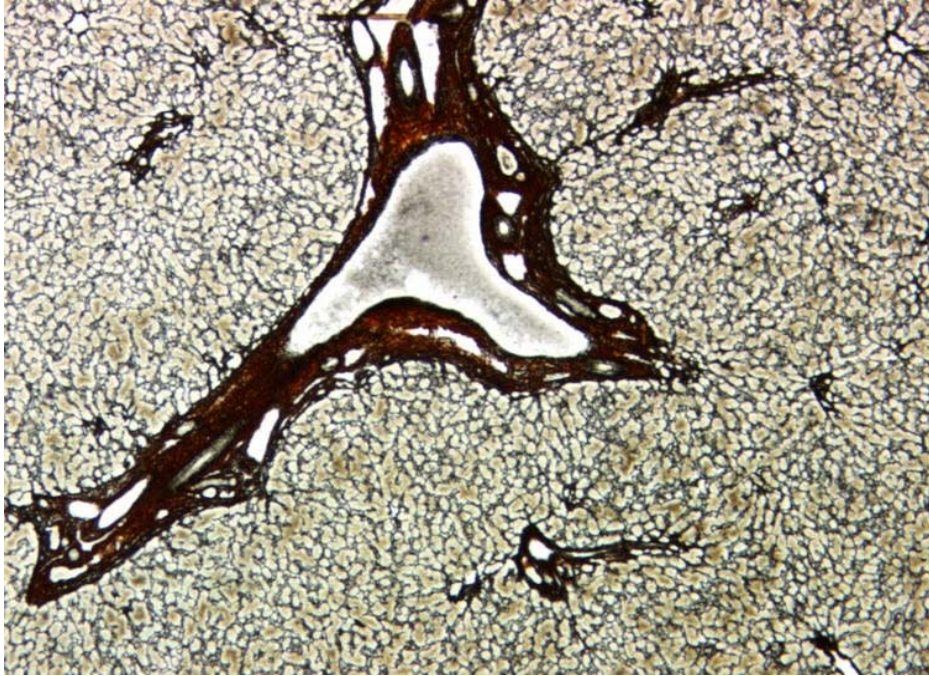


Liver 40X- PAS

Nucleus: blue

P.A.S. positive substance: magenta

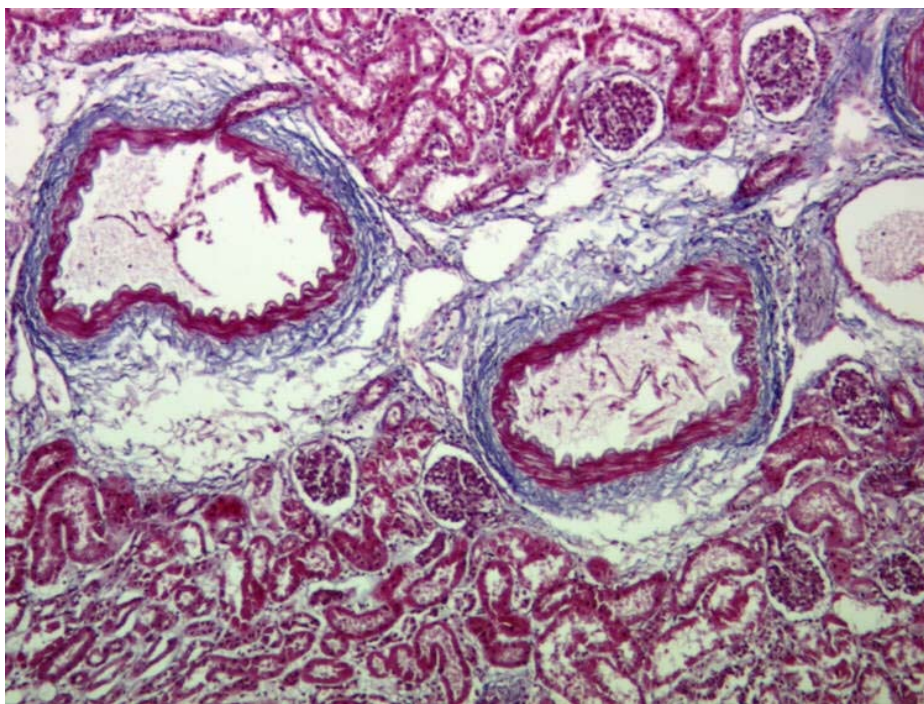
7.3 Silver Impregnation



Liver 5X – SILVER IMPREGNATION

Reticular and nervous fibers: black
Connective: brown
Collagen: yellow

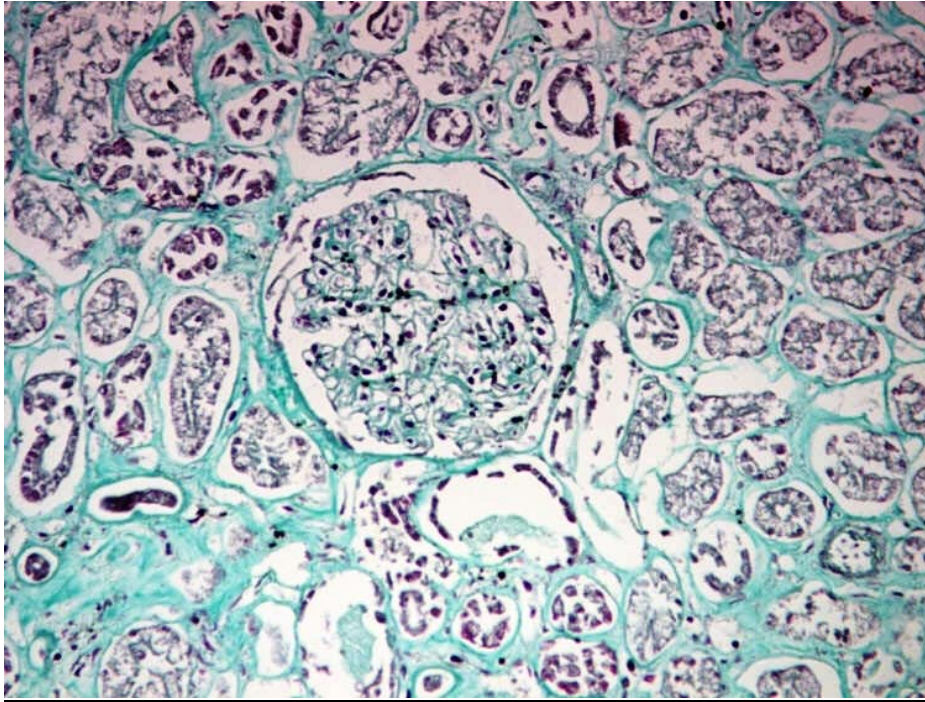
7.4 Masson trichrome acc. Capelli (with aniline blue)



Kidney 10X – MASSON TRICHROME ACC. CAPELLI (WITH ANILINE BLUE)

Nucleus: black
Collagen, mucus: blue
Muscular fibers, keratin, cytoplasm: high red

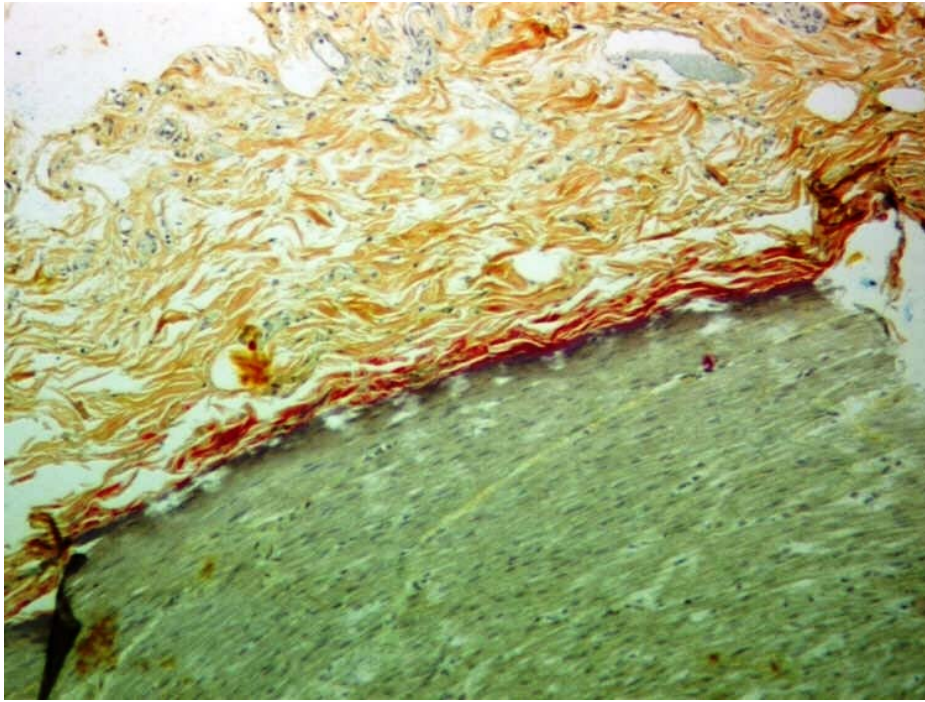
7.5 Goldner T (Masson trichrome with Light Green)



Kidney 20X – *GOLDNER T (MASSON TRICHROME WITH LIGHT GREEN)*

Nucleus: black
Collagen, mucus: green
Muscular fibers, keratin, cytoplasm: high red
Autoptic tissue

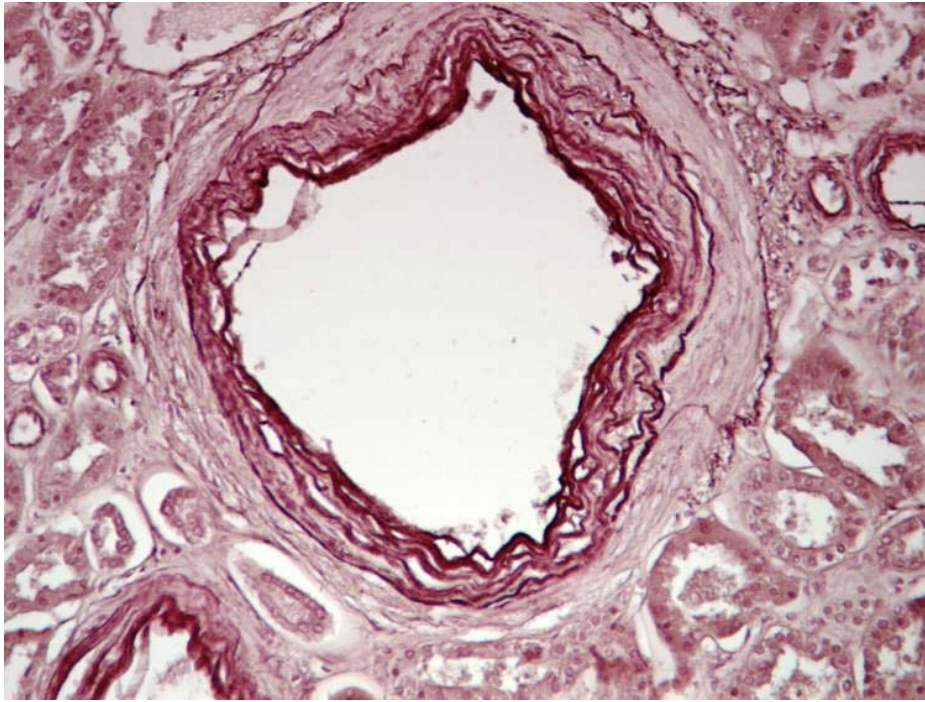
7.6 Movat Pentachromatic Stain



Intestine 10X – MOVAT PENTACHROMATIC STAIN

Nucleus and elastic fibers: black
Collagen and reticular fibers: yellow
Muscle: red

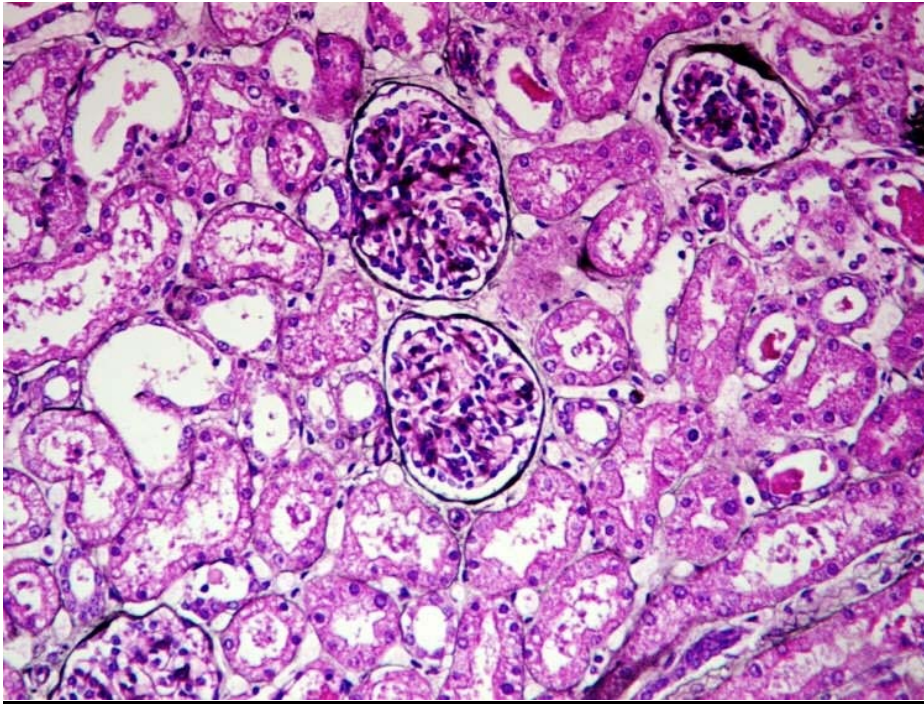
7.7 Acid Orcein



Kidney 20X – ACID ORCEIN

Elastic fibers: Dark brown

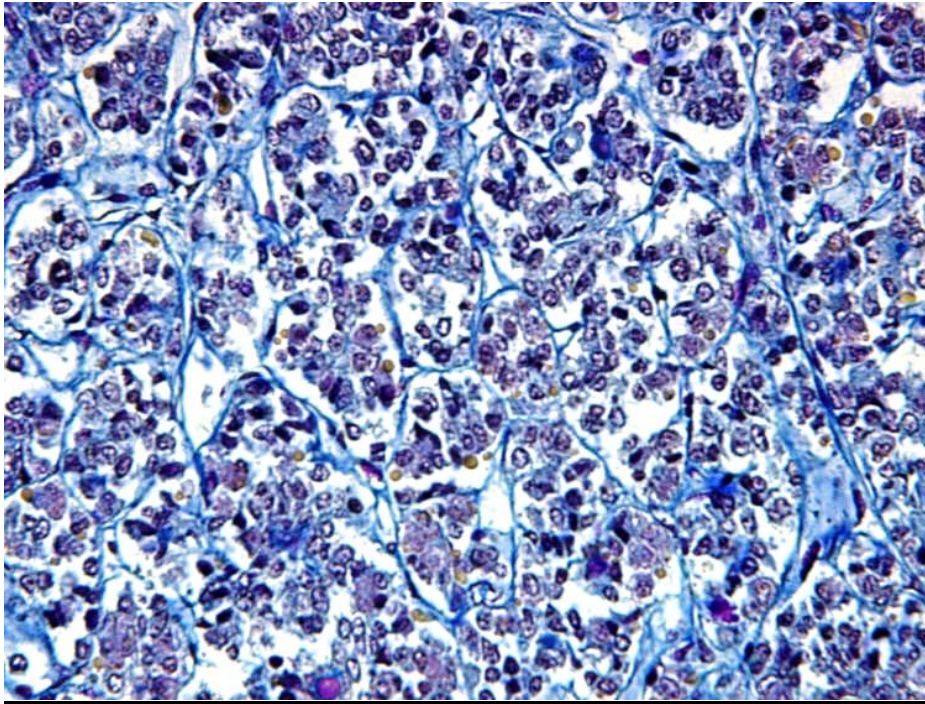
7.8 P.A.S.M.- Silver Methenamine acc. Callard



Kidney 20X – P.A.S.M. SILVER METHENAMINE ACC. CALLARD

Basal membrane: black

7.9 Picromallory trichrome acc. Lendrum



Hyphophysis 40X – PICROMALLORY TRICHROME ACC. LENDRUM

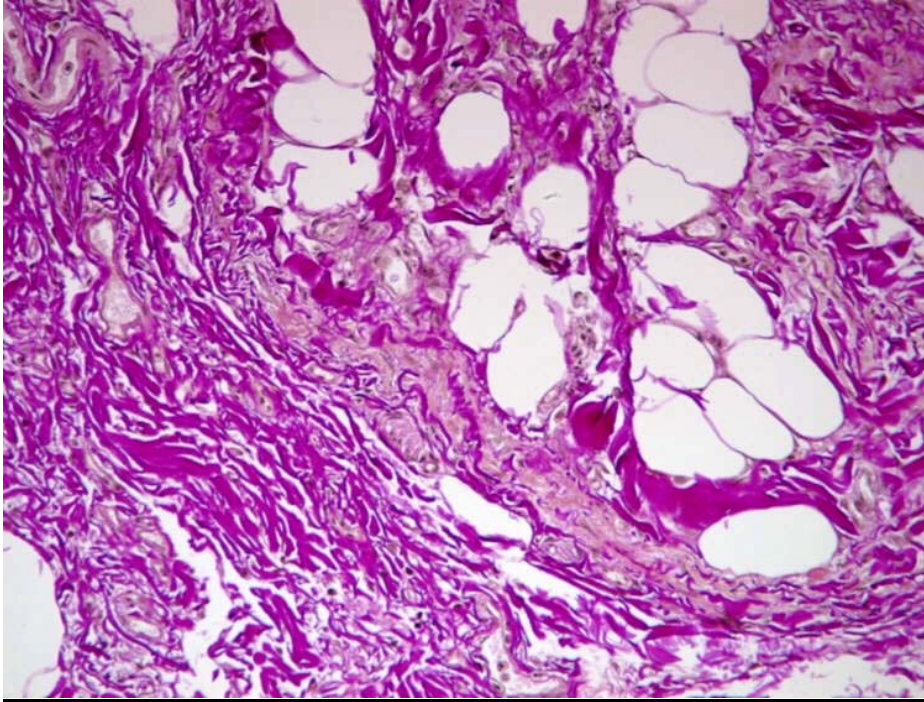
Nucleus: dark brown

Collagen fibers: dark blue

Hyphophysis basic substance: blue in different shades

Hyphophysis acidophil grains: orange

7.10 Van Gieson trichrome acc. Weigert



Intestine 20X – VAN GIESON TRICHROME ACC. WEIGERT

Nucleus: black

Collagen: red

Cytoplasm, epithelium horny stratus, muscle, erythrocytes: yellow

8.0 FAQ



WHAT IS GREENFIX?

GREENFIX is an histological fixative formaldehyde free. It is a colourless solution, not toxic or harmful, suitable for all kind of tissues fixation allowing formalin replacement in laboratories.

WHICH ARE GREENFIX COMPOUNDS?

GREENFIX is an aqueous solution with Ethandial and ethyl alcohol that allows a better fixative penetration into the tissue. Ethyl alcohol percentage is so low that it doesn't classify the product as flammable.

WHAT IS ETHANDIAL?

Ethandial is a dialdehydic molecule that, differently from formaldehyde, is odourless, less volatile and doesn't provoke physical troubles for the operator.

WHAT IS THE OPERATING MECHANISM?

Ethandial has a similar operating mechanism during aldehydic fixation as formaldehyde.

IS GREENFIX TOXIC?

No, its formulation has been intentionally studied to have the slightest impact for the user and the environment. GREENFIX is a non toxic or harmful solution. For further information see the Material Safety Data Sheet.

WHY CHANGE FORMALIN FIXATION?

The formaldehyde contained in formalin is a dangerous substance, classified as carcinogenic (IARC 2004), characterized by a pungent smell, much irritating to eyes and respiratory tract as well-know for Pathological Anatomy operators.

WHY CHOOSE GREENFIX?

Because it is a non toxic, non carcinogenic, odourless, formaldehyde and methanol free fixative and it doesn't provoke typical formalin unpleasant reactions (lachrymation and burning to eyes and respiratory tract). It doesn't demand any special working protocol changes too.

GREENFIX CAN BE USED IN ASSOCIATION WITH OTHER PATHOLOGICAL ANATOMY REAGENTS?

Of course, GREENFIX is compatible with standard laboratory reagents (alcohols, xylene or substitutes) and it can be used in histoprocessing first step. Any dilutions are necessary; the product is ready to use.

HOW USE IT?

As well as formalin; obviously it is possible to observe formalin "good practises" as following: respect ratio volume specimen/fixative; check fixation time; open and clean up organs before putting them into the fixative; etc.

GREENFIX is also suitable for specimens with small or medium dimensions. For further information see the operating manual.

WHAT IS THE DIFFERENCE BETWEEN GREENFIX AND GREENFIX PLUS?

The two formulations are both ready to use, non toxic or harmful.

GREENFIX PLUS has ethyl alcohol high concentration allowing ethandial penetration into bigger specimens. For further information see the Safety Data Sheet.

THE MORPHOLOGY WILL BE PRESERVED?

We have tested the fixative in different centres and asked to different pathologists; and all are amazed to see preparation high quality with a morphology comparable to formalin fixation: in particular it shows a better detail than chromatin. Special stains and Immunohistochemistry have done good results too.

The most evident artefact consists of blood cells are not so clear or they have an "invisible" appearance; however it has not hindered the diagnosis.

WHICH ARE THE RESULTS IN IMMUNOHISTOCHEMISTRY?

Immunohistochemistry results are good and comparable to formalin cases.

We suggest to not use a retrieval with protease, because it has a too aggressive effect on tissue.

HOW DISPOSE GREENFIX?

Always in accordance with local regulations concerning special waste disposal, GREENFIX is disposed as non toxic or harmful product; instead GREENFIX PLUS as flammable products (but non toxic or harmful).

Dr. Stefania Bencini
Martinengo, 09/12/2008

DIAPATH

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