Application Bulletin
Validated protocols

Tissue-Tek® VIP®6AI

Tissue Processing

Tissue-Tek® VIP® 6AI

General information
This bulletin contains background information on the validated & recommended protocols for VIP-6 AI and how to upload the into the instrument.

Processing protocols VIP® Vacuum Infiltration Processors
These protocols are validated and recommended by Sakura. The protocols are for further evaluation and validation in your own laboratory. No rights or claims can be made related to the results of the protocols as shown/mentioned in this bulletin.

Background information best quality results
For optimal results take into account:

1. The importance of correct collection and transportation of tissue samples. (Avoid drying out of tissue and use formalin in 1:20 ratio).
2. The importance of correct fixation of tissue samples.
3. The tissue should be grossed on a 2-3 mm thickness, don't overfill the cassettes.
4. In microtomy the following items are important to get optimal sections; the cooling of the blocks, clearance angle of the blade holder and the use of proper, sharp blades.
5. The hot plate for drying slides should not exceed temperatures of 40°C-45°C, a low temperature prevents nuclear bubbling.

More detailed information can be found in: Theory and Practice of Histological Techniques by John D Bancroft chapter 4 to 7 isbn 9780443102790

Reagents used:

Fixative: **Alcohol / Formalin** used to preserve the tissue, the structures of the cell, and the cell organelles found in the individual cells.
- 450 ml Formaldehyde 36%
- 4050 ml Ethanol 55%

Dehydration: **Ethanol** to remove all extractable water from the tissue.

Clearing: **Xylene or Tissue-Clear** as defatting solution and intermedium to remove dehydrant and sets up tissue for infiltration with paraffin.

Impregnation: **Paraffin** as impregnation agent, paraffin will solidify and subsequently support the cells/tissue.

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Mixtures:

Alcohol / Formalin

- In general tissue is fixed in formalin, which is an all-round fixative but doesn't react on lipids.
- Alcohol / formalin mixture is a good general fixative which gives an extensive extraction of lipids → removes fat from the tissue.
- Alcohol / formalin mixture affords a greater degree of tissue penetration than formalin alone
- When using an alcohol / formalin mixture in the first step of the processing protocol, dehydration and extraction of fat directly starts.

Alcohol / xylene / Tissue Clear

- Alcohol xylene mixtures facilitates an increased dehydrating and defatting
- Improvement of the paraffin infiltration
- Relatively short fatty tissue protocol with excellent results

References:

- *Theory and Practice of Histological Techniques by John D Bancroft*
- *Diagnostic histochemistry by Mark R. Wick*
- *Understanding Fixation, Ada Feldman*
- *http://philschatz.com/anatomy-book/contents/m46006.html*
- *Iwadare, T., Basic theories in dehydrating, defatting and paraffin-infiltrating fixed tissues, Pathology Technical Manual No.3, Pathology Specimen Preparation Technologies, Vol. #1, Ishiyaku-Shuppan, Tokyo, 1981; 63-76.*
- *Takeishi, M., Basic course for pathological techniques (3) Dehydration and defatting, Pathology and Clinical Medicine, 1988; 6(6):697-701.*
- *Kawashima, T., Evaluation of Immunoenzyme Method for Mammary Tumor No.1 Tissue Processing (centered on defatting methods), Pathology Technologies, 1991; Vol.44 (8):8-10.*
- *Nishikawa, T., et al, Paraffin infiltration effect of xylene/ethanol pre-dehydration on automated tissue processor, Japanese Journal of Medical Technology, 2011; Vol.60 No.6: 857-863.*
- *Ross MH, Pawlina W., Histology: A text and atlas. 5th ed., (Aiiso S., Uchiyama Y., A Japanese version of Histology, 2. Non-membranous organelles, Tokyo: Nanko-Do, 2010; 23-70)*
- *Uedaira, H., Chap.5 Water in Living Organisms, What is Water – Its Microscopic Actions, Kodansha, Tokyo, 2009: 142-177*
- *Tamura, T., Part I Basis for Cell Biology, Basic Cell Biology, Tokyo Kagaku Doujin, Tokyo, 2010; 2-42*
- *Fuji, H. editor, Chap. 2 Physical Chemistry of Protein Structured, A Guide of Protein Conformation, Kodansha, Tokyo, 2010: 18-52*

Validated & Recommended protocols Sakura

1. o/n processing, using xylene

Station	Reagent	Time	Temperature (°C)	P/V	Mix
1	Alc./Formalin	0:30	37°C	Yes	Cont.
2	Eth. 100%	0:30	RT	Yes	Cont.
3	Eth. 100%	0:30	RT	Yes	Cont.
4	Eth. 100%	0:45	RT	Yes	Cont.
5	Eth. 100%	0:45	RT	Yes	Cont.
6	Eth./xyl (2:1)	0:45	RT	Yes	Cont.
7	Eth./xyl (1:2)	1:00	RT	Yes	Cont.
8	Xylene	0:30	RT	Yes	Cont.
9	Xylene	0:45	RT	Yes	Cont.
10	Xylene	0:45	37°C	Yes	Cont.
11	Paraffin	0:30	63°C	Yes	Cont.
12	Paraffin	0:45	63°C	Yes	Cont.
13	Paraffin	1:00	63°C	Yes	Cont.
14	Paraffin	1:00	63°C	Yes	Cont.
Total		**10:00:00**			

Note: Pumping in and out takes 45 minutes

2. Biopsy protocol, using xylene

Station	Reagent	Time	Temperature (°C)	P/V	Mix
1	Alc./Formalin	0:30	40°C	Yes	Cont.
2	Eth. 100%	0:08	40°C	Yes	Cont.
3	Eth. 100%	0:09	40°C	Yes	Cont.
4	Eth. 100%	0:10	40°C	Yes	Cont.
5	Eth. 100%	0:10	40°C	Yes	Cont.
6	Eth./xyl (2:1)	0:10	40°C	Yes	Cont.
7	Eth./xyl (1:2)	0:15	40°C	Yes	Cont.
8	Xylene	0:08	40°C	Yes	Cont.
9	Xylene	0:10	40°C	Yes	Cont.
10	Xylene	0:15	40°C	Yes	Cont.
11	Paraffin	0:10	63°C	Yes	Cont.
12	Paraffin	0:10	63°C	Yes	Cont.
13	Paraffin	0:15	63°C	Yes	Cont.
14	Paraffin	0:20	63°C	Yes	Cont.
Total		**3:00:00**			

Note: Pumping in and out takes 45 minutes

Note: With the initial set-up of the configuration, it is not necessary to dilute the first Ethanol 100% in the group, station 2, as a mixture of Formalin and ethanol 50% is used in the first station.

When deviations are made on this protocol, a concentration of 80% Ethanol can be advised in the first station of the Ethanol group.

Refreshment rate:

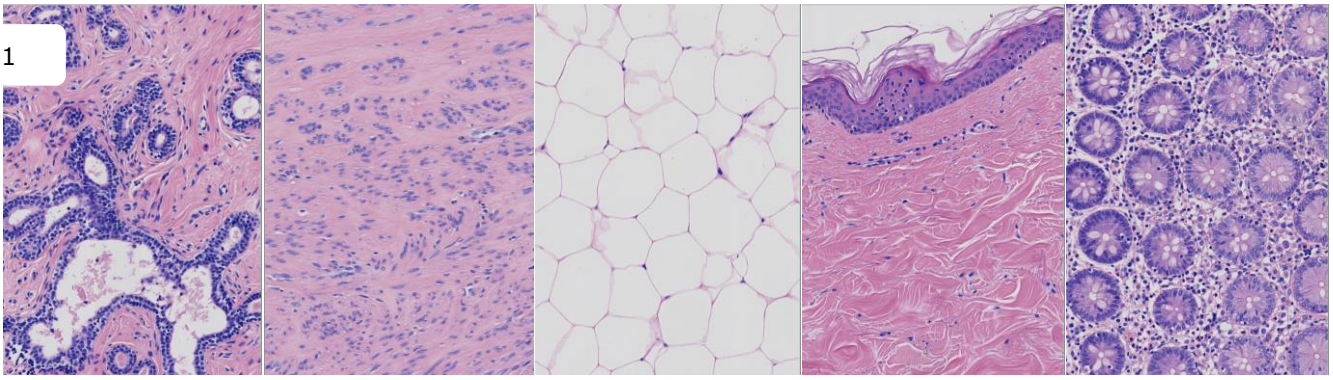
Based on a workload of 300 cassettes / day

Reagent		Refreshment rate
Alc./Formalin	➔	Once a week
Eth.100%	➔	First reagent bottle (station nr. 2) every 2 weeks via an automatic transfer during processing
Eth. / xyl. Mixtures	➔	Every 2 weeks
Xylene	➔	First reagent bottle (station nr. 8) every 2 weeks via an automatic transfer during processing
Paraffin	➔	First reagent bottle (station nr. 11) once a week via an automatic transfer during processing

Microscopic results xylene o/n processing (VisionTek® 20x):

Tissue Processing

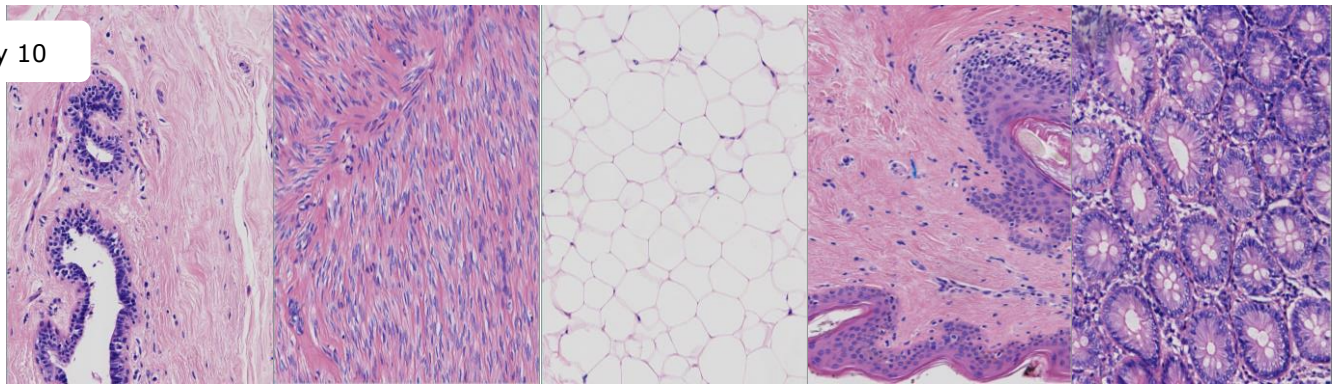
Day 1



Breast *Uterus* *Fat* *Skin* *Intestine*

Tissue-Tek® VIP® 6AI

Day 10



3. o/n processing using Tissue-Clear®

Station	Reagent	Time	Temperature (°C)	P/V	Mix
1	Alc./Formalin	0:30	37°C	Yes	Cont.
2	Eth. 100%	0:30	RT	Yes	Cont.
3	Eth. 100%	0:30	RT	Yes	Cont.
4	Eth. 100%	0:45	RT	Yes	Cont.
5	Eth. 100%	0:45	RT	Yes	Cont.
6	Eth./TC (2:1)	0:45	RT	Yes	Cont.
7	Eth.TC (1:2)	1:00	RT	Yes	Cont.
8	Tissue-Clear®	1:00	RT	Yes	Cont.
9	Tissue-Clear®	1:00	37°C	Yes	Cont.
10	Tissue-Clear®	1:30	40°C	Yes	Cont.
11	Paraffin	0:45	63°C	Yes	Cont.
12	Paraffin	0:45	63°C	Yes	Cont.
13	Paraffin	1:00	63°C	Yes	Cont.
14	Paraffin	1:15	63°C	Yes	Cont.
Total		12:00:00			

Note: Pumping in and out takes 45 minutes

4. Biopsy protocol, using Tissue-Clear

Station	Reagent	Time	Temperature (°C)	P/V	Mix
1	Alc./Formalin	0:30:00	40°C	Yes	Cont.
2	Eth. 100%	0:10:00	40°C	Yes	Cont.
3	Eth. 100%	0:08:00	40°C	Yes	Cont.
4	Eth. 100%	0:10:00	40°C	Yes	Cont.
5	Eth. 100%	0:08:00	40°C	Yes	Cont.
6	Eth./TC (2:1)	0:10:00	40°C	Yes	Cont.
7	Eth.TC (1:2)	0:15:00	40°C	Yes	Cont.
8	Tissue-Clear®	0:10:00	40°C	Yes	Cont.
9	Tissue-Clear®	0:15:00	40°C	Yes	Cont.
10	Tissue-Clear®	0:20:00	40°C	Yes	Cont.
11	Paraffin	0:12:00	63°C	Yes	Cont.
12	Paraffin	0:12:00	63°C	Yes	Cont.
13	Paraffin	0:15:00	63°C	Yes	Cont.
14	Paraffin	0:20:00	63°C	Yes	Cont.
Total		**03:12:00**			

Note: Pumping in and out takes 45 minutes

Note: With the initial set-up of the configuration, it is not necessary to dilute the first Ethanol 100% in the group, station 2, as a mixture of Formalin and ethanol 50% is used in the first station.

When deviations are made on this protocol, a concentration of 80% Ethanol can be advised in the first station of the Ethanol group.

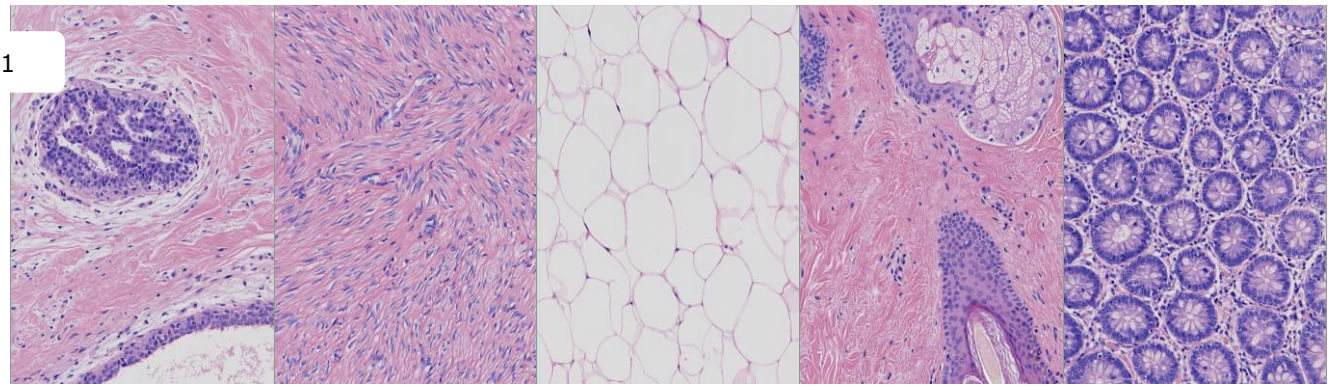
Refreshment rate:

Based on a workload of 300 cassettes / day

Reagent		Refreshment rate
Alc./Formalin	➔	Once a week
Eth.100%	➔	First reagent bottle (station nr. 2) once a week via an automatic transfer during processing
Eth. / Tissue Clear Mixtures	➔	Once week
Tissue Clear	➔	First reagent bottle (station nr. 8) once a week via an automatic transfer during processing
Paraffin	➔	First reagent bottle (station nr. 11) once a week via an automatic transfer during processing

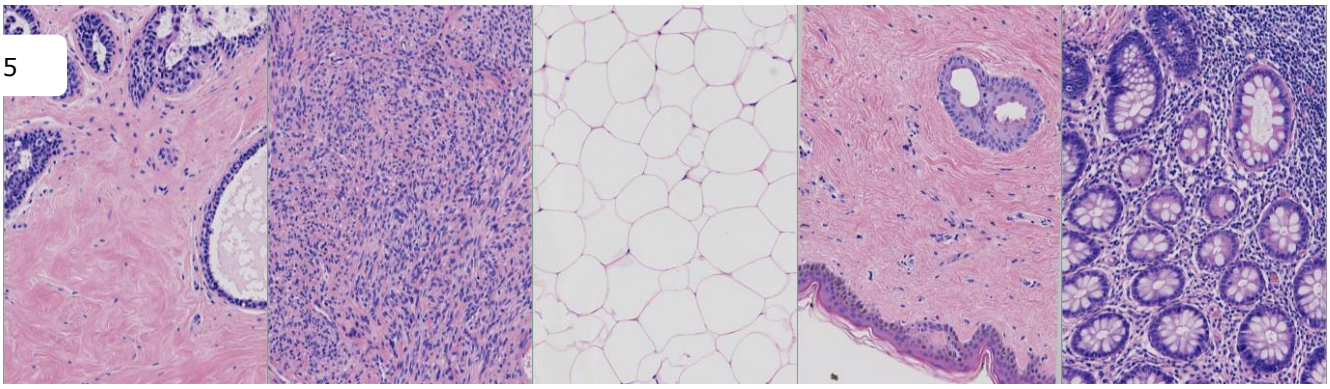
Microscopic results Tissue Clear o/n processing (VisionTek® 20x):

Day 1



Breast *Uterus* *Fat* *Skin* *Intestine*

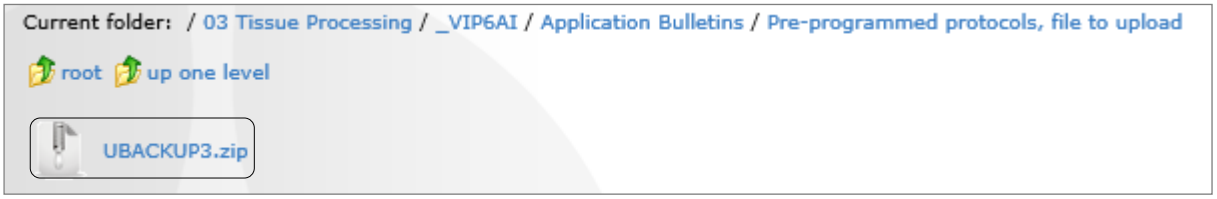
Day 5



Upload validated & recommended protocols:

- Validated & recommended protocols can be found on the DA in the VIP-6 AI folder / Application bulletins / Pre-programmed protocols, file to upload

- Save and "un-zip" the UBACKUP3.zip" folder on a USB key



- Log on to the VIP-6 AI with the service password
- Go to the Software / Data Update
- Place USB key with the unzipped "UBACKUP3" folder in the VIP-6 AI (no need to change the name)
- Press the "User Data Update 3" button
- Upload will start
- Instrument will restart directly after upload
- Remove USB key