ScyTek LABORATORIES INC.

Instructions For Use

HCS1014-IFU

205 South 600 West Logan, Utah 84323, U.S.A. – Tel. (800) 729-8350 – Tel. (435) 755-9848 – Fax (435) 755-0015 – www.scytek.com Rev. 1, 10/14/2024

Melanin Control Slides

Description
Melanin Control Slides contain formalin fixed paraffin embedded sections of Human Skin cut at 4 micron thickness. Sldies are known to contain Melanin and produce a positive staining result with Fontana-Masson Stain Kit.

Storage
Store slides at 2-25°C

Suggested Stain Kit *(not provided)*
FMS-1
See below procedure

Fontana-Masson Stain Kit

(For Argentaffin Cells and Melanin)

Description and Principle
The Fontana-Masson Stain Kit is intended for use in the histological visualization of Melanin and other argentaffin substances. Substances that can bind silver and reduce it to a visible metallic form without a separate reducing agent are said to be "argentaffin". Fontana-Masson stain has been reported to be useful in the identification of Capsule-Deficient *Cryptococcus neoformans* and typical *Cryptococcus neoformans*. Argentaffin granules and Melanin are demonstrated by silver impregnation using an Ammoniacal Silver Solution.

Expected Results

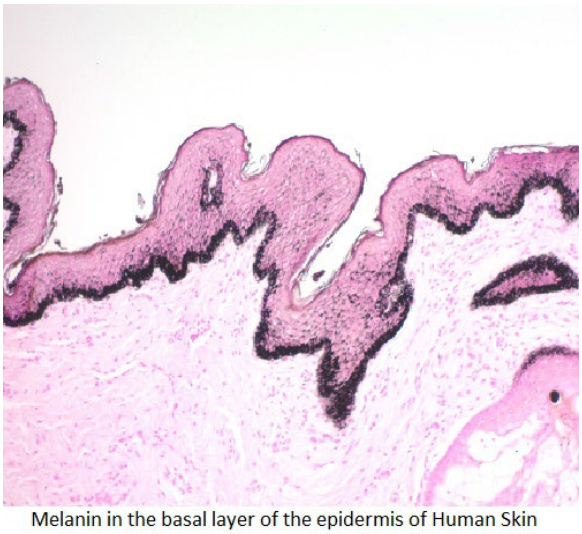
Argentaffin Cell Granules:	Black
Melanin:	Black
Cryptococci Cell Wall:	Black
Nuclei:	Red
Cytoplasm:	Light Pink

Kit Contents	**Storage**
1. Gold Chloride Solution (0.2%)	2-8°C
2. Silver Nitrate Solution (10%)	2-8°C
3. Sodium Thiosulfate Solution (5%)	18-25°C
4. Nuclear Fast Red Solution	18-25°C

Suggested Controls *(not provided)*
Tissue containing Hair Follicles or Skin for Melanin. Small Intestine or Appendix for Argentaffin Granules.

Uses/Limitations
For In-Vitro Diagnostic use only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage
Mixed storage conditions. Store according to individual label instructions.



Melanin in the basal layer of the epidermis of Human Skin stained with Fontana-Masson Stain. Magnification 100X

Safety and Precautions
Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

Required but not Included
Concentrated Ammonium Hydroxide Solution (25-30%)

Important Notes
1. All glassware used in this procedure should be chemically cleaned and rinsed thoroughly in distilled water.

2. Do not use metal forceps to remove slides from reagents. Use plastic forceps only.

3. Equilibrate all reagents to room temperature prior to use.

Preparation of Reagent Prior to Beginning:
Prepare **Ammoniacal Silver Solution** immediately prior to use.

In new or chemically cleaned glassware, mix 27ml Distilled/Deionized water with one vial of Silver Nitrate Solution (10%) and blend completely. Carefully add Concentrated Ammonium hydroxide (25-30%) (Not included) one drop at a time, swirling gently after each drop. Initially the mixture will turn dark brown and then gradually become transparent with a fine layer of sediment. The solution is ready for immediate use when all sediment dissolves.

Procedure
1. Deparaffinize sections if necessary and hydrate to distilled water.

2. Place freshly mixed Ammoniacal Silver Solution in a 58-60°C water bath and allow adequate time for temperature to equalize.

3. Incubate slide in warmed Ammoniacal Silver Solution for 30-60 minutes or until tissue section becomes yellowish/brown in color. (NOTE: Melanin typically stains in 30 minutes while Argentaffin granules stain in 50-60 minutes)

4. Rinse in 3 changes of distilled water.

5. Incubate slide in Gold Chloride Solution (0.2%) for 30 seconds.

6. Rinse in 3 changes of distilled water.

7. Incubate slide in Sodium Thiosulfate Solution (5%) for 1-2 minutes.

8. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.

9. Incubate slide in Nuclear Fast Red Solution for 5 minutes.

10. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.

11. Dehydrate very quickly in 3 changes of absolute alcohol.

12. Clear, and mount in synthetic resin.

ScyTek Laboratories, Inc.
205 South 600 West
Logan, UT 84321
U.S.A.

References

1. Kim HM, Byun K-A, Oh S, Yang JY, Park HJ, Chung MS, Son KH, Byun K. A Mixture of Topical Forms of Polydeoxyribonucleotide, Vitamin C, and Niacinamide Attenuated Skin Pigmentation and Increased Skin Elasticity by Modulating Nuclear Factor Erythroid 2-like 2. Molecules. 2022; 27(4):1276. https://doi.org/10.3390/molecules27041276
2. Lee, Eung-Ji, et al. "Whitening effect of novel peptide mixture by regulating melanosome biogenesis, transfer and degradation." The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology 25.1 (2021): 15-26. https://doi.org/10.4196/kjpp.2021.25.1.15
3. Kim, Ji-Hye et al. "JNK suppresses melanogenesis by interfering with CREB-regulated transcription coactivator 3-dependent MITF expression." Theranostics vol. 10,9 4017-4029. 4 Mar. 2020, doi:10.7150/thno.41502
4. Yun, Cheong-Yong et al. "Nuclear Entry of CRTC1 as Druggable Target of Acquired Pigmentary Disorder." Theranostics vol. 9,3 646-660. 21 Jan. 2019, doi:10.7150/thno.30276
5. Akimoto, K. , Yamaguchi, T. , Naraoka, Y. , Hu, A. and Kobayashi, H. (2019) Depigmentory Effects of Keishibukuryogankayokuinin in Human Epidermal Melanocytes. Health, 11, 869-879. doi: 10.4236/health.2019.117070.
6. Chang, Chung-Hsing, et al. "CK1α ablation in keratinocytes induces p53-dependent, sunburn-protective skin hyperpigmentation." Proceedings of the National Academy of Sciences 114.38 (2017): E8035-E8044. https://doi.org/10.1073/pnas.1702763114
7. H. Li, J. Kim, H.-G. Hahn, J. Yun, H.-S. Jeong, H.-Y. Yun, K. J. Baek, N. S. Kwon, Y. S. Min, K.-C. Park, and D.-S. Kim, "KHG26792 Inhibits Melanin Synthesis in Mel-Ab Cells and a Skin Equivalent Model," The Korean Journal of Physiology & Pharmacology, vol. 18, no. 3, p. 249, 2014.
8. H. Li, H.-Y. Yun, K. J. Baek, N. S. Kwon, K.-C. Park, and D.-S. Kim, "Myriocin, a serine palmitoyltransferase inhibitor, increases melanin synthesis in Mel-Ab cells and a skin equivalent model," Die Pharmazie - An International Journal of Pharmaceutical Sciences, vol. 69, no. 3, pp. 187-191, Mar. 2014.
9. C. M. O'Brien, K. D. Rood, K. Bhattacharyya, T. DeSouza, S. Sengupta, S. K. Gupta, J. D. Mosley, B. S. Goldschmidt, N. Sharma, and J. A. Viator, "Capture of circulating tumor cells using photoacoustic flowmetry and two phase flow," Journal of Biomedical Optics, vol. 17, no. 6, Jun. 2012.
10. T.-S. Chang and V. C.-H. Lin, "Melanogenesis Inhibitory Activity of Two Generic Drugs: Cinnarizine and Trazodone in Mouse B16 Melanoma Cells," International Journal of Molecular Sciences, vol. 12, no. 12, pp. 8787-8796, Dec. 2011.
11. V. C.-H. Lin, H.-Y. Ding, S.-Y. Kuo, L.-W. Chin, J.-Y. Wu, and T.-S. Chang, "Evaluation of in Vitro and in Vivo Depigmenting Activity of Raspberry Ketone from Rheum officinale," International Journal of Molecular Sciences, vol. 12, no. 8, pp. 4819-4835, Jul. 2011.
12. Sheenan, D.C., Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Battelle Press, Columbus, OH.
13. Gaitanis, G., et al. Novel application of the Masson-Fontana Stain for Demonstrating Malassezia Species Melanin-Like Pigment Production In Vitro and in Clinical Specimens. Journal of Clinical Microbiology. 2005, August; 43(8): pages 4147-4151.
14. Kimura, M., et al. Fontana-Masson – stained tissue from culture-proven mycoses. Archives of Pathology & Laboratory Medicine. 1998, December; 122(12): page 11.
15.Lazcano, O., et al. Combined Fontana-Masson-Mucin staining of Cryptococcus neoformans. Archives of Pathology & Laboratory Medicine. 1991, November; 115(11): pages 1145-1149.
16.Ro, J.Y., et al. Advantage of Fontana-Masson stain in capsule-deficient cryptococcal infection. Archives of Pathology & Laboratory Medicine. 1987, January; 111(1): pages 53-57.