

## AGR1000 - UA-Zero<sup>®</sup> EM Stain

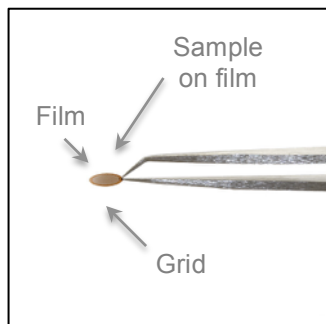
### Fixation, staining and processing of HeLa\* cells grown on coverslips

#### General directions for use of UA-Zero EM Stain

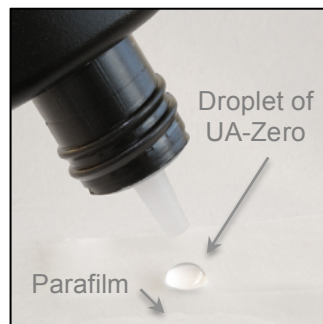
UA-Zero<sup>®</sup> EM Stain is a patented solution developed as a direct replacement for Uranyl Acetate. UA-Zero does not contain any radioactive material and is non-toxic. UA-Zero can be used as a direct substitute to Uranyl Acetate with no changes to standard user protocols.

UA-Zero is supplied in an opaque dropper bottle, which should be stored long-term in the lab fridge at around 4°C. For daily use, the sealed bottle can be stored in normal laboratory conditions away from direct sunlight.

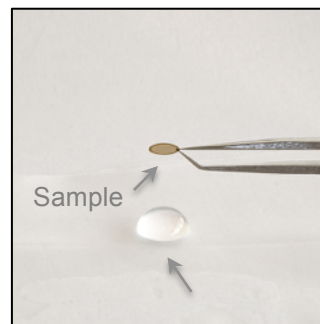
It has been shown that UA-Zero EM Stain works very well for various protocols that specify Uranyl Acetate as a staining solution for TEM sample preparation in life science. This application note describes one example of a protocol for preparing samples and subsequent imaging using TEM. Uranyl Acetate and UA-Zero EM stain were directly compared and the images generated are shown in the Results section.



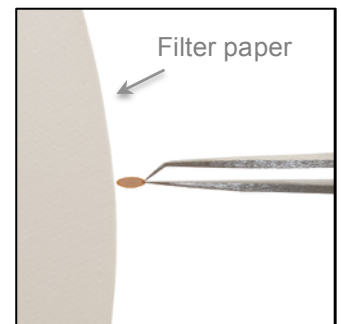
Sample loading.



A drop of UA-Zero EM Stain on Parafilm (AGG398).



Sample staining. Sample in contact with staining solution only.



Drain with filter paper.

Staining the sample on a TEM grid, with a support film, requires just a few simple steps before drying.



*\* HeLa cells are derived from cervical cancer cells taken on February 8, 1951 from Henrietta Lacks, nowadays being the oldest and most commonly used human cell line in scientific research.*

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## Protocol used for TEM sample preparation

All steps performed at room temperature unless otherwise stated. Parts of the same sample have been stained using UA-Zero EM Stain and Uranyl Acetate, for direct comparison.

- |   |             |
|---|-------------|
| 1. <b>Fixation:</b> 2.5% glutaraldehyde (AGR1012) in 0.1M cacodylate buffer | 1 hour      |
| 2. 0.1M cacodylate (AGR1105) washes   | 3 x 10 mins |
| 3. 1% OsO <sub>4</sub> (AGR1021) in 0.1M cacodylate buffer                  | 20 mins     |
| 4. 0.1M cacodylate washes   | 3 x 10 mins |
| 5. Deionised water  | 3 x 10 mins |
| 6. <b>Staining:</b>   |             |
| 6a UA-Zero EM Stain in 20% ethanol  | 1 hour      |
| 6b 3% Uranyl Acetate in water   | 1 hour      |
| 6c 3% Uranyl Acetate in 20% ethanol   | 1 hour      |
| 7. <b>Dehydration:</b> 70% ethanol  | 5 mins      |
| 8. 80% ethanol  | 5 mins      |
| 9. 90% ethanol  | 10 mins     |
| 10. 100% ethanol  | 3 x 10 mins |
| 11. <b>Infiltration:</b> 'Epon' Resin mix*: 100% ethanol, 1:1               | 1.5 hours   |
| 12. 'Epon' Resin mix  | 1.5 hours   |
| 13. <b>Embedding:</b> in 'Epon' Resin cure at 60°C                          | Overnight   |

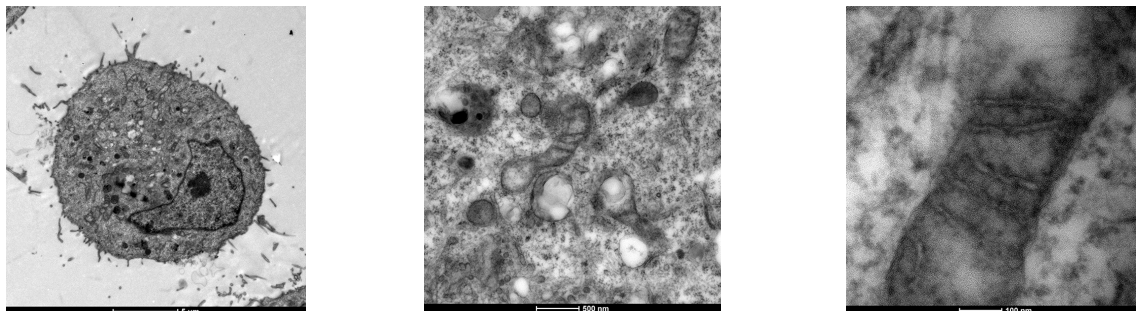
Sections cut the following day (**when resin not completely hard**) and picked up on pioloform copper slot grids. No further staining of sections. For better section quality, embedding should be done for 2 days.

\* 'Epon' type resin mix:

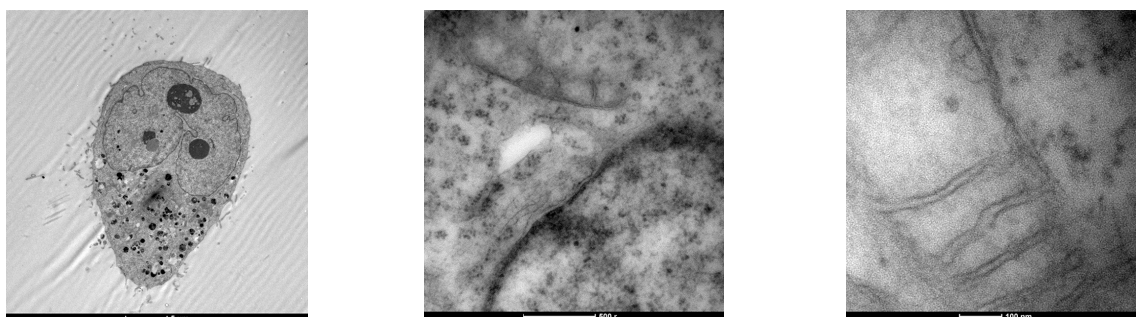
- 24g AGR1045 Agar 100 Resin
- 16g AGR1053 DDSA (Dodecenyl Succinic Anhydride) EM grade redistilled
- 10g AGR1083 MNA (Methyl Nadic Anhydride) redistilled
- 1.5g AGR1062 BDMA (Benzyl Dimethylamine)

## Results

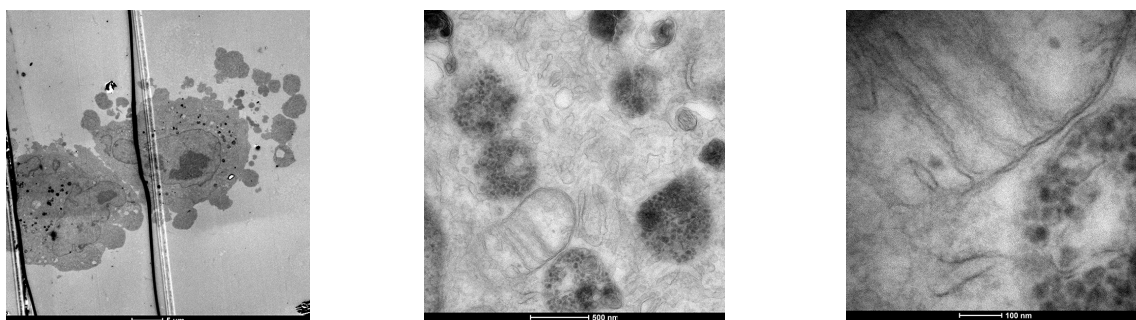
6a) TEM images of sample prepared using UA-Zero EM Stain in 20% ethanol as staining solution



6b) TEM images of sample prepared using 3% Uranyl Acetate in water as staining solution



6c) TEM images of sample prepared using 3% Uranyl Acetate in 20% ethanol staining solution



*Samples prepared and imaged courtesy of Prof. Dr. Paul Verkade and Dr. Chris Neal, Wolfson Bioimaging Facility, School of Medical Sciences, University of Bristol.*