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Whole-body clearing of beetles by successive treatment of hydrogen peroxide and CUBIC reagents

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1 Works for me [dx.doi.org/10.17504/protocols.io.ba33igqn](https://doi.org/10.17504/protocols.io.ba33igqn)

Optical Clearing of Tissue



ABSTRACT

Internal tissues of multicellular organisms cannot directly be seen because they contain pigments. For this reason, whole-body clearing methods have been developed and applied to mammals such as mice. Insects such as beetles, however, cannot be cleared by the mammalian method because of pigments such as melanin in their exoskeletons. In this study, we tried to develop a whole-body clearing method for large beetles. We first bleached the exoskeleton using a hydrogen peroxide treatment, and applied the the advanced Clear, Unobstructed Brain/Body Imaging Cocktails and Computational analysis (CUBIC) reagents to make the internal tissues transparent. The combined method of hydrogen peroxide and the advanced CUBIC allowed us to successfully perform whole-body clearing of the large beetles.

ATTACHMENTS

[Monto Kuroda.pdf](#)

MATERIALS TEXT

10% Formaldehyde Neutral Buffer Solution (Nacalai Tesque, cat. no. 37152-51)
4% Paraformaldehyde Phosphate Buffer Solution (FUJIFILM Wako Pure Chemical Co. cat. no. 163-20145)
Ethanol (FUJIFILM Wako Pure Chemical Co. cat. no. 057-00451)
Hydrogen Peroxide (H₂O₂) (FUJIFILM Wako Pure Chemical Co. cat. no. 081-04215)
Urea (FUJIFILM Wako Pure Chemical Co. cat. no. 219-00175)
N,N,N',N'-tetrakis (2-hydroxypropyl) ethylenediamine (Quadrol) (Tokyo Chemical Industry, cat. no. T0781)
Triton X-100 (Sigma-Aldrich, cat. no. X100-500ML)
Nitrilotriethanol (FUJIFILM Wako Pure Chemical Co. cat. no. 145-05605)
Sucrose (FUJIFILM Wako Pure Chemical Co. cat. no. 196-00015)



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