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Whole-body clearing of beetles by successive treatment of hydrogen peroxide and CUBIC reagents V.1

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Monto Kuroda[1], Shinya Kuroda[2]

[1]Keika High school, I, 5-6-6 Hakusan, Bunkyo-ku, 112-8612, Tokyo, Japan;
[2]Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Optical Clearing of Tissue

Shinya Kuroda
University of Tokyo

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Protocol status: Working
We use this protocol in our group and it is working.

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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

PDF

Monto Kuroda.pdf

408KB

Materials

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 (H_2O_2) (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-hydroxypropy- l) 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)

Troubleshooting