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Protein isolation from the extract of coelomic fluid of the Lumbricus terrestris.

Angel A Justiz-Vaillant¹

¹University of the West Indies St. Augustine

University of the West Indies

angel.vaillant@sta.uwi.edu

Angel A Justiz-Vaillant

University of the West Indies St. Augustine

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We use this protocol and it's working

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- 1 Six earthworms, Lumbricus terrestris, after removal from the earth are placed in a 15 ml glass tube and washed thoroughly with PBS, pH 7.4.

- 2 The body of the annelid is pierced with an 18-gauge needle.
- 3 The coelomic fluid content of the annelid is added to 2 ml of PBS, pH 7.4.
- 4 After incubation at 4°C for 1h, 0.9 ml chloroform is added.
- 5 The mixture is centrifuged at 1000 x g for 5 min at 4°C.
- 6 The supernatant (1.1 ml) is collected and equal volume of cold ethanol is added drop wise to the preparation.
- 7 The mixture is then incubated at 4°C for 12 h.
- 8 Then, it is centrifuged at 4°C for 5 min.
- 9 The pellet (protein extract) is resuspended in 0.3 ml of PBS, pH 7.4.

- 10 The preparation is dialyzed against 1L of PBS, pH 7.4 for 12 h at 4°C.
- 11 The protein concentration is assessed by the Bradford method or any available method.