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

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**Protocol status:** Working
**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$^{o}$C for 1h, 0.9 ml chloroform is added.

5 The mixture is centrifuged at 1000 x g for 5 min at 4$^{o}$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$^{o}$C for 12 h.

8 Then, it is centrifuged at 4$^{o}$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$^{o}$C.

11 The protein concentration is assessed by the Bradford method or any available method.