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Western Blotting (Fly Heads) V.2

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol describes how to perform a Western Blotting technique using fly heads.

Troubleshooting

1 Homogenize desired number of fly heads in 1 X Laemmli sample buffer.

2 Heat samples to 100 °C for 00:10:00 , spin briefly before loading. 10m

3 Load premade gel into western blotting apparatus. Fill reservoir with Running Buffer:
Running Buffer:
6 g Tris-HCL
28.9 g glycine
Fill to 1 L with distilled water
Add 5 mL 20% SDS

4 Load samples on gel and attach electrodes.

5 Run gel at 120 V until dye front reaches the bottom of the gel, 01:00:00 . Run longer for greater separation. 1h

6 Remove gel and transfer using Trans-Blot Turbo.

7 Perform antigen retrieval by microwaving 00:09:00 in PBS. 9m

8 Block membrane in 1X PBS with 0.05% Tween-20 and 3% dry milk for 01:00:00 . 1h

9 Add primary antibody at correct dilution in PBSTween + milk and incubate with shaking Overnight at 4 °C .

10 Wash blot 3x in PBSTween, 00:05:00 each, with shaking. 5m

11 Add secondary antibody at the correct dilution in PBSTween + milk, incubate with shaking at Room temperature for 03:00:00 . 3h

12 Wash blot in PBSTween 00:30:00 with frequent wash changes. 30m

13 Develop with ECL substrate or image fluorescence, as appropriate.