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Preparation of cigarette smoke extract (CSE)

Antioxidants

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Works for me

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Notes

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- All procedures should be done in a fume hood to minimize exposure of the experimenter and laboratory environment

to the cigarette smoke.

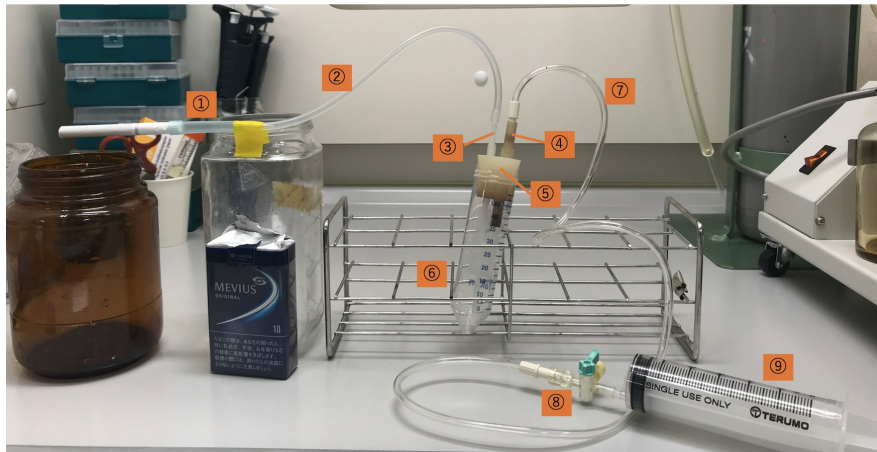
- The CSE should be prepared immediately before use.

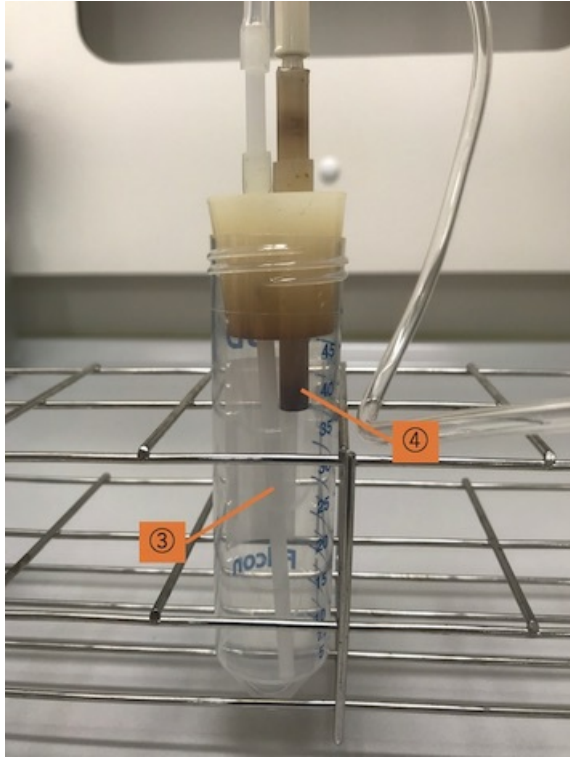
Materials

1. 1000 µl pipette tip
2. Silicone tubing (Tigers Polymer, SR-1554, inner diameter: 3 mm, outer diameter: 5 mm, length: 250 mm)
3. Polypropylene tubing (inner diameter: 2 mm, outer diameter: 4 mm, length: 150 mm)
4. Polypropylene tubing (inner diameter: 5 mm, outer diameter: 7 mm, length: 75 mm)
5. Silicone stopper with two holes
6. 50 ml polypropylene conical tube (Thermo Fisher Scientific, 339657)
7. Extension tubing (Terumo, SF-ET5527, 750 mm)
8. Three-way stopcock (Terumo, TS-TR2K)
9. 50 ml syringe (Terumo, SS-50LZ)
10. phosphate-buffered saline (PBS)
11. Millex-LG sterilizing filter unit (Merck/Millipore, SLLG025SS, 0.2 µm)
12. Cigarettes

Protocol

- 3 Assemble the smoking apparatus.





- 4 Fill the 50 ml polypropylene conical tube with 10 ml of PBS (6), and cap it with the silicone stopper (5).
- 5 Attach the 50 ml syringe (9) to the 3-way stopcock (8) connected to the extension tubing (7).
- 6 Insert the cigarette into the 1000 µl pipette tip (1), and attach it to the silicone tubing (2).
- 7 Light the cigarette while pulling on the syringe plunger.
- 8 Draw the syringe plunger to the 50 ml mark in 10 seconds (flow rate: 300 ml/min). The mainstream smoke is bubbled through PBS in the conical tube.
- 9 Turn the stopcock off to the extension tubing and push the plunger back into the syringe.
- 10 Open the stopcock to the extension tubing.

- 11 Repeat the steps 8-10 until the cigarette burns to the filter (typically 10 to 11 times per cigarette).
- 12 Remove the cigarette butt and insert the new cigarette to the 1000 µl pipette tip for the next round of extraction.
- 13 Repeat the procedure for a total of 5 cigarettes (smoke from 5 cigarettes is bubbled through 10 ml of PBS).
- 14 Filter the extract through the 0.2 µm pore size membrane, and the resulting solution is defined as 100% CSE.