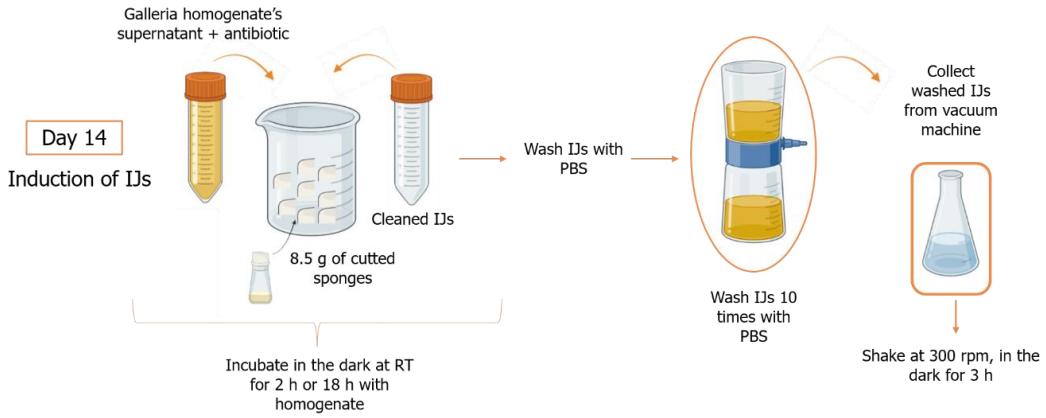

Figure 7. Protocol scheme of day 14: **Induction of IJs using sponge pieces.**

2.6.2. Liquid induction

The liquid induction protocol was performed according to Toubarro et al. [24]. Around 1 million fourteen-days-old *H. bacteriophora* IJs were washed 3 times with filtered water in a vacuum filter with 10 µm filters as previously described. Forty mL of PBS was added to the washed IJs and they were transferred into a 10 cm Petri dish with 4 mL of induction material (in the case of non-induced (NI) condition, PBS was used as an induction material), 30 µL of Kanamycin (50 mg/mL) and 30 µL of Streptomycin-Penicillin (10000 U/mL) (Figure 8). The Petri dish was incubated shaking at 100 rpm, at RT, in the dark for a specific amount of (2h or 18 h) (Figure 6, See Annex 3).

Subsequently, the IJs were washed 10 times with PBS in the vacuum filter holder previously mentioned with two layers of 10 µm filters (Figure 8). In a Petri dish, IJs were mixed with 40 mL of PBS, as well as 30 µL of Kanamycin (50 mg/mL) and 30 µL of Streptomycin-Penicillin (10000 U/mL). The dish was incubated shaking at 100 rpm at RT, in the dark, for 3 h.

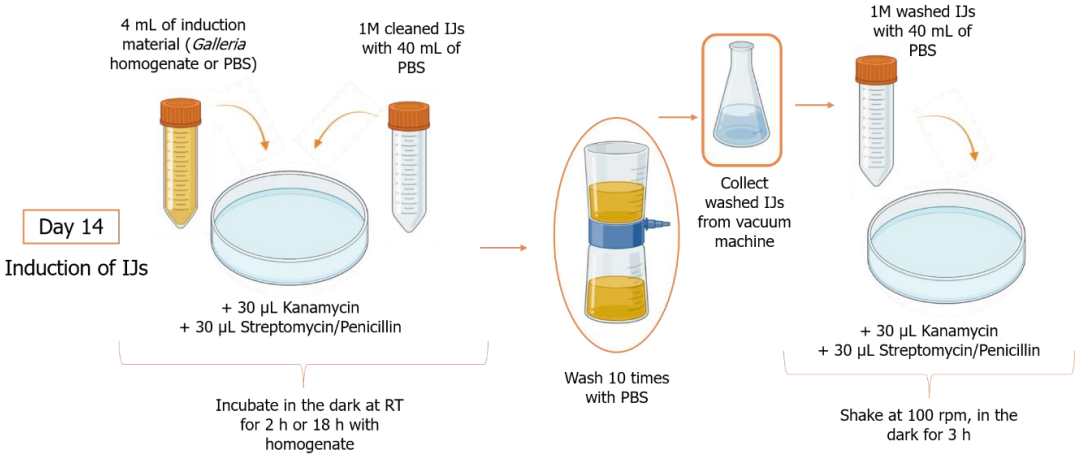

Figure 8. Protocol scheme of day 14: **Induction of IJs using the liquid protocol.**

2.7. Collection of ESPs

The IJs, which were kept for excretion/secretion of ESPs, were filtered with plastic vacuum filter and a 0.2 µm cellulose acetate membrane filter OE66 (Whatman, Great Britain) to collect the ESPs. The obtained suspension was kept on ice. The ESPs were concentrated to approximately less than 200 µL using an Amicon Ultra 15 mL centrifugal 3 kDa filter (Merck Millipore, Ireland) by centrifugation using a swing-bucket rotor at 5000 rcf, 4ºC, 45-135 min (Figure 9). The concentrated ESPs were immediately used for injection or stored at -80ºC until future use.

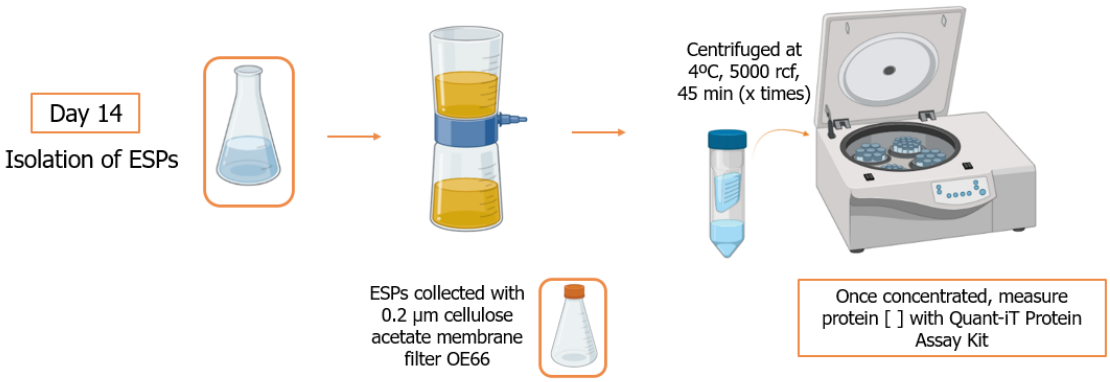

Figure 9. Protocol scheme of day 14: **Collection of ESPs.**

2.8. Protein concentration measurement of ESPs

After concentrating the ESPs, their protein concentration was measured using Invitrogen Quant-iT Protein Assay Kit (Invitrogen, USA). Firstly, the assay components were equilibrated at RT, followed by the preparation of the working solution by diluting Quant-iT protein reagent in Quant-iT protein buffer 1:200. Then, 200 µL of working solution was added in each microplate well, in addition to 10 µL of Quant-iT protein standard or protein sample. Once the samples were well mixed and incubated in dark for 15 minutes at RT, the fluorescence was measured at 470/570 nm with spectrophotometer Sense (Hidex, Finland).

2.9. ESPs toxicity assay

Two weeks old flies were sorted to separate males and females. After 24-72 h, the sorted male flies were anesthetized with CO_2 and injected intra-thoracically (Figure 10) with a volume of 50 nL of ESPs or PBS as a negative control. The maximal protein concentration of obtained ESPs was injected, being 0.04 µg/µL the minimal and 0.29 µg/µL the maximal concentration injected.