

Substrate preparation

Substrate plate

The plate is sealed and stored frozen until use

Substrate suspension (100 μ l) is dispensed into flat bottom 96-well polypropylene plate using pre-cut 1 ml carbon tips

Plate thawing

Fungi secretome preparation

Reducing sugar releasing assay

Glucose scale addition (125 μ l)

Enzyme addition (25 μ l)

Plate sealing. Transfer to shaking incubator

24 - 72 h at 37 or 50°C with 8Hz orbital shaking

Robot punches seal with needles and adds 125 μ l of 50 mM Na acetate pH5

Suspension is allowed to settle for 5'

PCR plate is sealed and transferred to heating block

60 μ l DNS reagent are added to a polypropylene PCR plate, followed by 60 μ l of filtered reaction mixture**

Upper 125 μ l are uptaken and vacuum filtered through 1 μ m* into polystyrene plate**

10' at 94°C then 5' at room temperature

Seal is pierced, and the content resuspended by aspiration; 100 μ l are then transferred to a polystyrene plate.

The polystyrene plate is transferred to the microplate reader for OD₅₄₀ recording

The amount of released sugar is calculated using glucose reference scale