

## **Sucrose Assay Kit**

(Sigma, Product code SCA-20)

### Sucrose extraction

- add 400  $\mu$ l 80% (v/v) Ethanol to 100 mg frozen ground tissue
- incubate for 15 min at 78°C on a thermomixer
- centrifuge at 14000 rpm, 5 min, 4°C
- transfer supernatant to a new tube (2 ml)
- re-extract pellet for two times with 500  $\mu$ l 50% (v/v) Ethanol (same procedure)
- pool all subfractions and evaporate samples
- resuspend in 1 ml water
- dilute until 100-1000  $\mu$ g sucrose/ml (Assay-Result:  $\Delta A_{340} = 0.03-1.6$ )

### Assay

- Standard curve: prepare 1mg/ml sucrose solution  
dilute to 100  $\mu$ g, 200  $\mu$ g, 500  $\mu$ g, 800  $\mu$ g, sucrose
- prepare the following blanks:

| Tube                | Sucrose Assay reagent (ml) | Sample Volume (ml) | Water (ml) |
|---------------------|----------------------------|--------------------|------------|
| Sucrose Assay blank | 0.1                        | --                 | 0.1        |
| Sample blank        | --                         | 0.1                | 0.1        |
| Glucose Assay blank | --                         | --                 | 0.2        |
| Test                | 0.1                        | 0.1                | --         |

- mix and incubate for 10 min at room temperature
- add 2ml of the Glucose Assay Reagent to each tube
- mix and incubate for 15 min at RT
- Measure absorbance at 340 nm

for 1/10 of volume

| Tube                | Sucrose Assay reagent (ml) | Sample Volume (ml) | Water (ml) |
|---------------------|----------------------------|--------------------|------------|
| Sucrose Assay blank | 0.01                       | --                 | 0.01       |
| Sample blank        | --                         | 0.01               | 0.01       |
| Glucose Assay blank | --                         | --                 | 0.02       |
| Test                | 0.01                       | 0.01               | --         |

- mix and incubate for 10 min at room temperature
- add 0.2ml of the Glucose Assay Reagent to each tube
- mix and incubate for 15 min at RT
- Measure absorbance at 340 nm