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Assay of anthocyanin biosynthetic enzyme activity

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Preparation of buffer

1 25 mM HEPES buffer (pH 7.4) is preparated with EDTA (0.2 mM), AsA (2 mM), and PVP (2%), which was kept in -4℃ refrigerator.

Leaf tissue sample extraction

2 0.2 g of leaf samples were ground in 2 ml of ice-cold 25 mM HEPES buffer (pH 7.4) ,The homogenates were centrifuged at 4°C for 20 min at the speed of 12,000 r.p.m, and removed supernatants to assay the enzymatic activities.of *CHS*, *CHI*, *F3H*, *DFR*, *ANS* and *ANR*, using an ELISA Kit (U.S.A TSZ biological Trade Co., Ltd.) according to the manufacturer's instructions.

Add standard

3 Set stantard wells, testing sample wells. Add standard 50 µl to standard well.

Add sample

4 Set blank wells separately (blank comparson wells don´t add sample and HRP-Conjugate reagent, other each step operation is same). Testing sample well. add sample dilution 40 µl to testing sample wells, then add testing sample 10 µl (sample final dilution is 5-fold), add sample to wells, don´t touch the well wall as far as possible, and mix gently.

Incubate

5 After closing plate with closure plate membrane, incubate for 30 min at 37℃.

Comfigurate liquid

6 30-fold wash solution diluted 30-fold with distiled water and reserve.

Washing

7 Uncover closure plant membrane, discard liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.

Add enzyme

8 Add HRP-Conjugate reagent 50 µl to each well, except blank well.

Incubate and washing

9 Incubate is operated with step 5, and washing is operated with step 7.

Color

10 Add chromogen solutio A 50 µl and chormogen solution B to each well, evade the light preservation for 15 min at 37℃.

Stop the reaction

11 Add stop solution 50 µl to each well, stop the reaction (the blue color change to yellow color).

Assay

12 Take adding stopbland well as zero, read absorbance at 450nm after adding stop solution and within 15 mim