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In vitro human tyrosinase inhibitory assay (human melanoma cell lysate based)

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We use this protocol and it's working

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Abstract

Tyrosinase (TYR) inhibitors are required to treat skin hyperpigmentation. Currently live cell-based TYR assays and mushroom TYR *in vitro* assays are the common methods used to screen for TYR inhibitors. However, these methods are either time consuming and expensive or are not human TYR (*hs*TYR) specific. Here, we describe a simple *hs*TYR assay using cell lysate prepared from pigmented human melanoma cell lines that takes less than 3 hours to complete after collecting cell pellets. We confirmed the assay is species specific by using a known *hs*TYR inhibitor, kojic acid, as a positive control, while arbutin, which inhibits mushroom TYR, but not *hs*TYR, was not effective. This assay is a simple method to confirm *hs*TYR inhibition before conducting follow-up studies in live biological models.

Materials

Cell lines and materials
MM418C1 is a clone of the MM418 melanoma cell line that originated from the primary lesion of the cutaneous surface (Clark *et al.*, 1994 & Fechner *et al.*, 1994).

Reagents
L-Glutamine, penicillin/streptomycin, trypsin, RPMI 1640 medium (powder) were purchased from Life Technologies (CA, USA). Bovine serum albumin (BSA), 3,4-Dihydroxy-L-phenylalanine (L-Dopa), sodium phosphate, kojic acid, Triton X-100 and T75 flask were purchased from Sigma Chemicals (NSW, Australia). Fetal bovine serum (FBS) was purchased from Bovogen Biologicals (VIC, Australia). Trypan blue 0.4% solution was purchased from MP biomedicals (VIC, Australia). The DC protein assay kit was purchased from Bio-Rad (NSW, Australia)

Safety warnings

Proper PPE must be worn all the time.
Risk assessment for handling chemicals and equipment use must be undertaken.

In vitro human tyrosinase inhibitory assay (human melanoma cell lysate based)

2h 25m

1 Seed human melanoma MM418C1 cells (or equivalent melanoma cell line) in a T75 cell culture flask and grow with RPMI 1640 medium, which contains 10% (v/v) fetal bovine serum (FBS), 200 mM L-glutamine, 100 U/mL penicillin, and 100 g/mL streptomycin (Sigma chemicals, Australia) until 80% confluent.

2 Mix 10 µL of cell suspension with 10 µL of 0.4% (w/v) trypan blue solution, then apply 10 µL of the mixture onto a clean hemocytometer and count the cells. 10m

3 Collect 3 × 10^6 cells (can be stored at -80 °C for up to at least 672:00:00 without loss of tyrosinase activity) by trypsinization and centrifugation at 300 x g, 25°C, 00:05:00 . 10m

4 Lyse the cell pellet using 600 µL Triton X-100 lysis buffer (1% (v/v) Triton X-100, 50 mM sodium phosphate buffer, pH 6.8 , Sigma chemicals, Australia) and sonicate for 00:00:30 On ice using Epishear Sonicator (CA, USA). 5m

5 Collect the supernatants by centrifugation at 7500 x g, 4°C, 00:30:00 . 30m

6 Determine the protein concentration of the clarified cell lysate with the DC protein assay kit (Bio-Rad, Australia) 15m

7 For each sample, 20 µL of the potential tyrosinase inhibitors (1 mg/mL) were added to a 96-well plate in triplicate, followed by 160 µL of 15 mM L-3,4-dihydroxyphenylalanine(L-Dopa) solution in 0.1 M sodium phosphate buffer (0.049 M $Na_2HPO_4•7H_2O$, 0.051 M $NaH_2PO_4•H_2O$). Finally, 20 µL of the clarified cell lysate was added to each well. 10m

7.1 20 µL of 1 mg/mL kojic acid + 160 µL L-DOPA + 20 µL cell lysate is used as the positive control,

20 µL of 1% (v/v) Triton X-100 lysis buffer + 160 µL L-DOPA + 20 µL cell lysate is used as the blank.

8 Measure the change in absorbance at λ475 nm over 01:00:00 using a CLARIOstar multi-mode plate reader (BMG Labtech, Germany) and normalize the data by using the protein concentration obtained in step 6. 1h

9 Tyrosinase activity (% remaining) can be calculated using the following equation: 5m

Tyrosinase activity (%) = [(A1-A2) / (A3-A4)] * 100%

L-DOPA solution and sodium phosphate buffer were used as a blank (A1)
L-DOPA and cell lysate without test sample were used as a negative control (A2)
L-DOPA and cell lysate with the test sample were the test reactions (A3)
L-DOPA and the test sample without cell lysate were used as a negative control (A4)

Protocol references

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