**Editorial comments:**  
Changes to be made by the Author(s):  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

**Response**: We carefully reviewed the manuscript. Thanks.

2. Unfortunately, there are a few sections of the manuscript that show overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please revise lines: 62-69, 71-75,

**Response**: We revised the manuscript comply with editor’s comments.

3. Please define the error bars in all of the figures: SD, SEM, etc.

Response: all data were expressed as the mean **±** SEM**.**

4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

**Response:** We revised 3.2 section in text. Eppendorf tube to microcentrifuge tube.

5. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

**Response**: We revised the manuscript in response to the reviewer’s comment.

6. 2.2: What test compounds are used and at what concentrations? We need specific examples.

**Response:** We used arbutin (125 μg/mL) for inhibitor control. And we revised 2.2 section in text.

7. 2.3: How is the treatment done?

**Response**:We revised 2.3 section in text.

8. Please provide the composition of all solutions used.  
**Response:** We added 1.11 and 1.12 section in text.   
**Reviewers' comments:**  
  
**Reviewer #1:**   
Manuscript Summary:  
In the summary author must add the usefulness of these protocol over other available protocols  
**Response:** We added brief information in the text (line 366-369).

There are several

Major Concerns:  
In the discussion section authors must compare the protocols with other protocols available before claiming that these protocols are more reliable, cost effective ,handy and rapid.  
Precautions must be incorporated at the end of each protocol.  
**Response**: The method described in the manuscript is very common and widely used method in this field, which is also cost effective, handy, and rapid. We added this information in the manuscript (line 366-369).

Minor Concerns:  
In the manuscript there is repetition of some paragraphs (Line 27 to 29 and 78 to 81 ) that should be removed.

**Response:** We revised lines 80-81 in the text**.**

In the protocols section language should be more clear and understandable (Lines 119,123,127,129,146)

**Response:** We revised methods in the text (please see sentences in yellow highlights).

Legend of figure should be more informative  
**Response**: We revised the figure legends.

**Reviewer #2:**   
Manuscript Summary:  
Three standard methods for evaluating the hypopigmentation activity of chemicals are clearly described in this manuscript. Biochemical procedures and image analysis, which are both researcher-friendly methods, are shown here. I believe this manuscript is useful for researchers.  
  
Minor Concerns:  
-Line 88-91 should be revised to correctly refer Cooksey et al (5). Because it suggests that an immediate metabolite of L-tyrosine produced by tyrosinase-catalyzed oxidation is dopa-quinone.

**Response**: According to Cooksey et al., they mentioned that there has been some controversy in the literature regarding the method of generation of DOPA. DOPA is formed directly by the hydroxylation of tyrosine, whereas DOPA is formed indirectly.

According to the indirect theory, dihydroxy derivatives involve formation by nucleophilic attack on dopaquinone, either by external nucleophiles (e.g. thiols such as cysteine) or by nucleophilic groups attached to the quinone as in the case of DOPA, where the side chain amino group acts as an intramolecular nucleophile. The nucleophilic property of the amino group is due to the lone pair electrons on the nitrogen, and attack on the ring by the amino group is followed by re-aromatization, involving hydrogen transfer to give the corresponding catechol as shown in Reaction 1. Evidence from pulse radiolysis experiments (5) indicates that 5,6-dihydroxyindolene (cyclodopa) reacts rapidly with dopaquinone to give rise to two products, DOPA and dopachrome (Reaction 2). Thus, according to this scheme, DOPA in essence arises by disproportionation of dopaquinone (DQ), i.e. 2 DQ3DOPA 1 dopachrome, and is not a direct product of tyrosine hydroxylation by tyrosinase. If Reaction 2 is correct, it should be possible

-Procedure written in line 163 does not give 0.1 % solution.

**Response**: We revised 1.10 section in text.

-Line 179 Reason why FBS and 1 % P/S is removed should be described.

**Response**: There are several ways to treat cell. In this protocol, we tried to exclude unexpected effect of treatment. The presence of undefined constituents in serum may enhance or suppress the effect of the tested drug or toxins. In addition, antibiotics can occur competition effects to test compound. So we routinely used serum free medium without antibiotics for cell treatment.

-Line 181 Method for measuring cellular tyrosinase activity written here will cause misunderstanding. The effect of tested materials on the down-regulation of tyrosinase expression cannot be excluded based on this method. Direct inhibition of tyrosinase enzyme should be clearly separated from it.

**Response**: We changed the title as in vitro assay.

-Line 198 The optional procedure is performed to what? Addition of the enzyme will hide the contribution of intracellular tyrosinase.  
**Response**: We agree with your opinion but B16F10 cells are unstable to produce melanin when cells are confluent.