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# ABS Orange Polyphenols PBF Efficacy Data

**Code:** 16734
**INCI Name:** Water & Propylene Glycol & Citrus Aurantium Amara (Bitter Orange) Flower Extract
**CAS #:** 7732-18-5 & 57-55-6 & 72968-50-4
**EINECS #:** 231-791-2 & 232-387-9

| Type of Study | Results |
|---|---|
| **PDE Inhibition Study** | An *in-vitro* study was conducted to look at **ABS Orange Polyphenols PBF** and its ability to inhibit phosphodiesterase (PDE). It was found **that ABS Orange Polyphenols PBF** is capable of inhibiting PDE thus promoting the mobilization of stored fats to reduce the appearance of dimply skin. |
| **ORAC Assay** | Oxygen Radical Absorbance Capacity (ORAC) is a measure of a materials potential to protect against oxidative stress or reactive oxygen species (ROS). **ABS Orange Polyphenols PBF** demonstrated significant antioxidant activity by reducing the presence of ROS compared with Trolox, the vitamin E analog. |



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# ABS Orange Polyphenols PBF PDE Inhibition Study

**Code:** 16734
**INCI Name:** Water & Propylene Glycol & Citrus Aurantium Amara (Bitter Orange) Flower Extract
**Suggested Use Levels:** 1.0 - 10.0%


## Abstract

Phosphodiesterase III (PDE) is a rate limiting enzyme that is responsible for the conversion of cAMP to 5'-AMP, thus favoring lipolysis by increasing intracellular cAMP concentration. Lipolysis, which is stimulated by cAMP, is the breakdown of lipids and involves the hydrolysis of triglyceides into free fatty acids. **ABS Orange Polyphenols PBF** is a lipolytic active ingredient that inhibits the activity of PDE and catalyzes the production of cAMP, thereby promoting the mobilization of stored fats.


## Materials and Methods

Inhibition was measured in-vitro using a biochemical model based on liquid scintillation quantification of the conversion of cAMP to 5'-AMP by purified human phosphodiesterase III. **ABS Orange Polyphenols PBF** was tested at 1%, 3% and 5%.

## Results

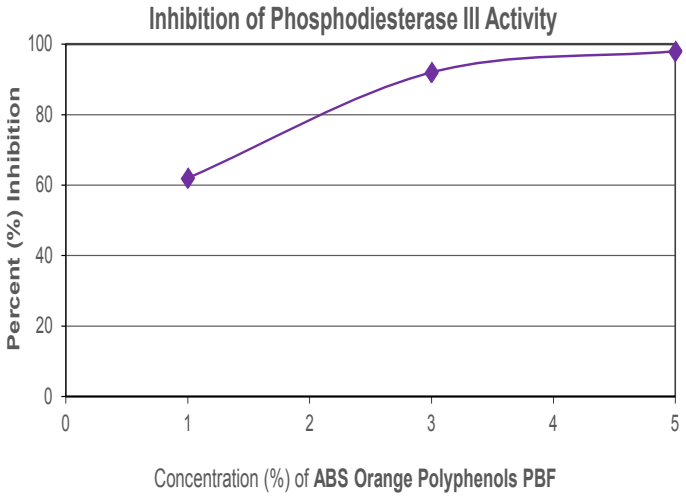


Figure 1. Results of PDE activity when **ABS Orange Polyphenols PBF** was tested in-vitro.

## Discussion

**ABS Orange Polyphenols PBF** is a lipolytic ingredient that is capable of inhibiting the activity of PDE thus stimulaing lipolysis. **ABS Orange Polyohenols PBF** was found to significantly inhibit PDE activity by 62%, 92% and 98% when tested at 1%, 3% and 5%.




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# Oxygen Radical Absorbance Capacity (ORAC) Assay

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**Tradename:** ABS Orange Polyphenols PBF

**Code:** 16734

**CAS #:** 7732-18-5 & 57-55-6 & 72968-50-4

**Test Request Form #:** 91

**Lot #:** NC120514-D

**Sponsor:** *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
**Study Director:** *Erica Segura*
**Principle Investigator:** *Meghan Darley*

**Test Performed:**
Oxygen Radical Absorbance Capacity (ORAC)

## Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are detrimental to cellular structures and functional molecules (i.e DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **ABS Orange Polyphenols PBF**.

## Assay Principle

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.

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## Materials

| | |
|---|---|
| **A. Equipment:** | Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes |
| **B. Buffers:** | 75mM Potassium Phosphate (pH 7.4); Deionized $H_2O$ |
| **C. Reagents:** | 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4nM) |
| **D. Preparation:** | Pre-heat (37°C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH. |
| **E. Microtitre Plates:** | Corning 96 Well Black Side/Clear Bottom Microplates |

## Methods

Solutions of **ABS Orange Polyphenols PBF** and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at a concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37˚C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software using the below equations:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow Where\ R\ is\ fluorescence\ reading$$

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample's Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents (µMTE), where 1 ORAC unit is equal to 1 µMTE.

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## Results

**ABS Orange Polyphenols PBF** began exhibiting antioxidant activity at a 0.05% concentration.

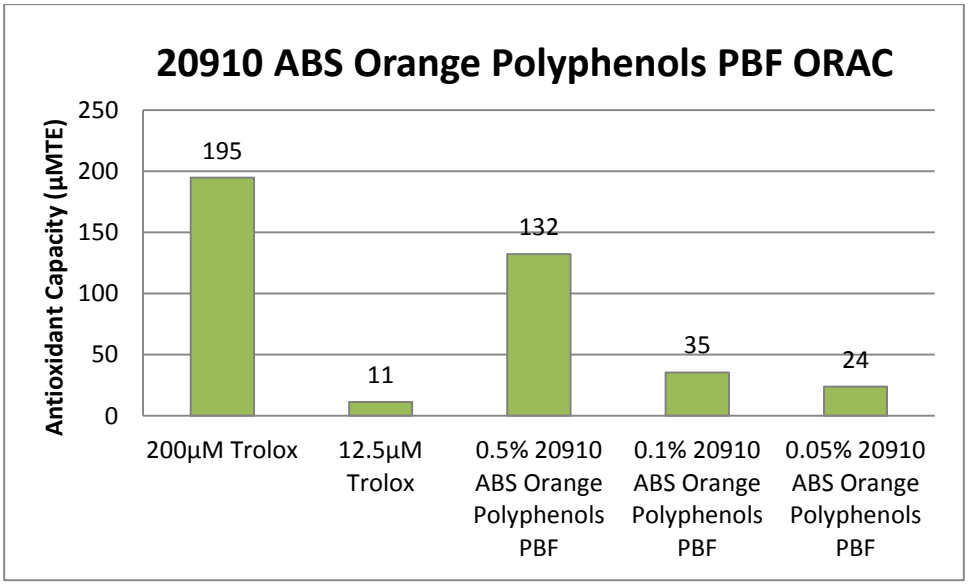


**Figure 1**: Antioxidant capacities

## Discussion

As shown in figure 1, **ABS Orange Polyphenols PBF** exhibited antioxidant activity comparable to our known antioxidant, Trolox®. The antioxidant capacity of **ABS Orange Polyphenols PBF** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

**ABS Orange Polyphenols PBF** was designed to provide anti-cellulite properties and function as a slimming agent. With the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic applications.