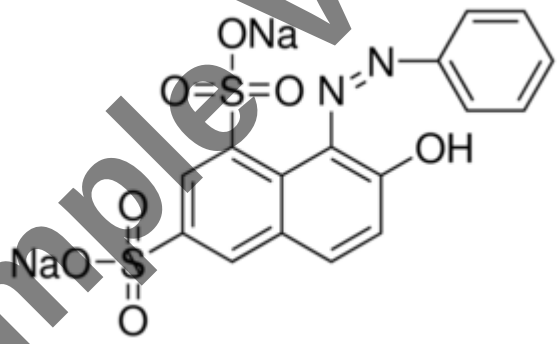
Keratin Orange

α-Keratin Assay

for hair



Molecular Structure of Keratin Dye (Orange G)

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life science assays

α-Keratin Assay Protocol for Hair

α-Keratin abbreviated to α-K

Solubilisation of Hair - Wash hair sample in water, followed by methanol. Air dry at room temperature until constant weight.

Add digestion reagent (1ml) to hair sample (5mg).

Leave to sit for 1 hour, then vortex or triturate with a 200µl pipette. This allows all the hair to disintegrate.

Standard Preparation - Dissolve 5mg reference standard in 1ml digestion buffer. Dilute this standard, in digestion buffer, to give 200µl standards of the following concentrations – 0,1,2,3,4 and 5mg/ml.

Neutralisation Step - Mix digested sample (200µl) and standards (200µl) with 1MHCl (200µl) in microcentrifuge tubes. Run duplicate tubes of all standards and samples.

Dye Binding - To each standard and sample add dye reagent (50µl), mix with vortex and leave to react for 30 minutes.

Salting Out of Keratin - Add saturated ammonium sulphate solution (450µl) to the 450µl neutralized sample/standard dye solutions. Microcentrifuge at 12000rpm for 10 minutes. Pour off the supernatant (the supernatant contains the unreacted dye).

Wash - To remove unbound dye, soluble protein and soluble protein cross reacted with the dye add 750µl water to each standard / sample and vortex. Leave to sit for 10 minutes. Microcentrifuge at 12000rpm for 10 minutes. Pour off supernatant and tap tube on tissue to remove dye droplets stuck to side of tube. Discard all wash.

Solubilisation of Keratin - To break down sulphides and keratin add 250µl of digestion buffer. Vortex until completely dissolved.

Measurement - Transfer 200µl of each sample and standard to individual wells of a 96well plate. Read plate in microplate reader at 480nm against air.

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EXTRACELLULAR MATRIX ASSAYS

for life sciences

Measure soluble and insoluble collagen, glycosaminoglycans, elastin and hyaluronan.

The Sircol™ family of Collagen Assays are based on a dye-binding method for the analysis of either insoluble collagens or acid and pepsin-soluble collagens. Soluble assay S1000 – 120 assays. Insoluble assay S2000 – 100 assays *(other kit sizes available)*.

Biocolor's Fastin™ Elastin Assay kit is a quantitative dye-binding method for the analysis of elastins extracted from mammalian sources. Measure soluble tropoelastins, lathyrogenic elastins and insoluble elastins following solubilization to elastin polypeptides (α-elastin and κ-elastin). Fastin F2000 (110 assays) *(other kit sizes available)*

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Blyscan™ is Biocolor's dye-binding method for the analysis of sulfated proteoglycans and glycosaminoglycans, (sGAG). Test material can be assayed directly when present in a soluble form, or following papain extraction from biological materials. Use to measure total sGAG content or adapt to determine the O- and N-sulfated glycosaminoglycan ratio. Blyscan B1000 (110 assays) *(other kit sizes available)*.

Biocolor's Purple-Jelley is designed to measure hyaluronan found in mammalian tissue. This is a dye-binding method which uses the dye 'Stains-all'. The method details the removal of tissue protein and sulfated glycosaminoglycans before measuring the isolated HA, from a twostep critical electrolyte salting out process (CEC). Purple-Jelley H1000 (100 assays) *(other kit sizes available)*.

Biocolor's other assays:

Cell-Clock Cell Cycle Assay – detection and measurement of cell cycle phases (C1000)
Cell-APOPercentage Apoptosis Assay – to measure apoptosis in mammalian cells (A1000)

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Keratin Orange
α-Keratin Assay

The Keratin Orange Assay has been designed for research work only.
Handle the Keratin Orange Assay using Good Laboratory Practice.

TECHNICAL INFORMATION

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Nature of Keratin

α-keratin is a fibrous structural protein found in all vertebrates in hair, wool, stratum corneum of the skin, horns, nails, claws and hooves. It is a unique protein due to its insolubility in water. Its strength is due to a high sulphur content. Strong disulphide bonds make it insoluble in water and resistant to most chemical agents. The amino acid composition varies depending on the tissue in which it occurs.

Keratin Orange Kit Pack Sizes and Storage Conditions

Standard Assay Kit Product Code: K1000 (110 assays)
Economy Pack Product Code: K5000 (440 assays)

All components are stable for 6 months (from Invoice Date) when stored at 15-25˚C. The glass vial containing keratin standard should be stored at +4˚C once opened.

Assay Kit Components

1. **Dye Reagent** contains Orange G in H_20 and has been formulated for specific binding to keratin under the conditions defined in the Keratin Orange Manual.

2. **Digestion Buffer** contains 100mM sodium hydrogen carbonate, 1% sodium thioglycolate and sufficient sodium hydroxide in deionised H_2O to bring pH to 11.5.

3. **Reference Standard** - a sterile solution of keratin in digestion buffer (5.0mg/ml) within a sealed vial.

   Store at 4˚C, remove aliquots using a sterile needle and discard if the clear solution becomes turbid.

4. **Neutralising Solution** 1M HCl

5. **Saturated Solution of ammonium sulphate**

Components required for sample preparation - not supplied

6. **Wash**, phosphate buffered saline

Equipment Required

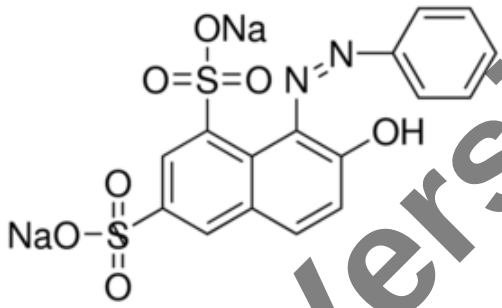
A centrifuge, fitted with a 1.5ml microcentrifuge tube rotor head; capable of 10,000 x g.

A microplate Reader, with a suitable colour filter (absorbance peak of dye occurs at 480nm).

INTENDED APPLICATIONS

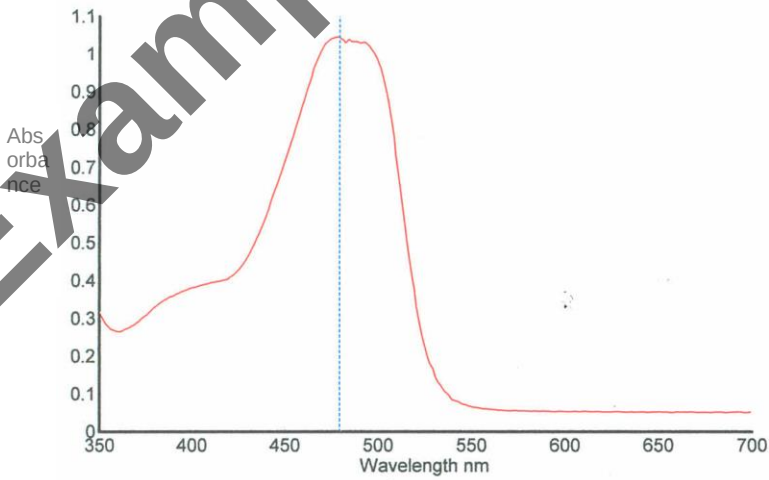
The assay has been designed for use with mammalian hair samples only. It may be suitable for use with animal tissue but this has not been verified.

KERATIN ORANGE DYE STRUCTURE

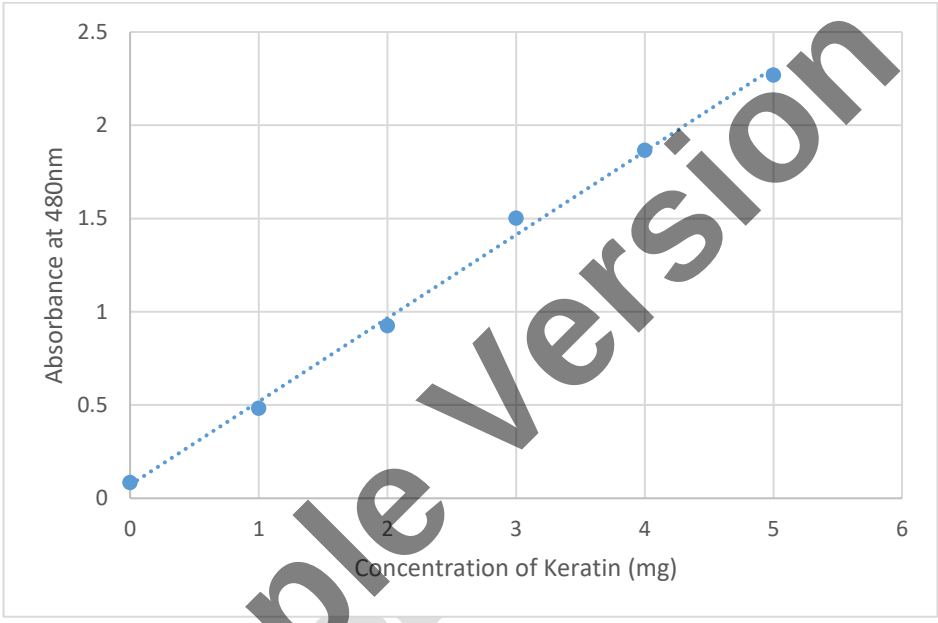


Molecular structure of Keratin Orange Dye (Orange G)

ABSORBANCE SPECTRUM OF KERATIN ORANGE DYE



STANDARD CURVE



Typical Straight Line Standard Curve for Keratin Reference Standards.

Microplate readers vary in their design and performance so this figure should be considered as a guide only.