

# Eppendorf Amber Conical Tubes: Maximal Sample Protection and Visibility

Rafal Grzeskowiak<sup>1</sup>, Sandrine Hamels<sup>2</sup>, Blandine Vanbellinghen<sup>2</sup>

<sup>1</sup>Eppendorf AG, Hamburg, Germany

<sup>2</sup>Eppendorf EAT, Namur, Belgium

## Abstract

Handling and storage of light-sensitive reagents or samples is commonly performed in amber-colored plastic tubes, which reduce or completely block light transmission in the short wavelength UV/VIS spectrum range. While providing good sample protection, the majority of amber tubes have however a major drawback: they are opaque and do not allow direct sample visibility. In this Application Note, protection of the sample from light (fluorescein recovery assay) and sample visibility (transmission spectrum) in several amber conical tubes were investigated. All tubes tested showed good protection of light sensitive samples (nearly 100% fluorescein recovery rates), which was confirmed by very low (close to 0%) light transmission in the wavelength range between 200 nm and approximately 550 nm. The Eppendorf Amber Conical Tubes are the only tubes that are moderately transparent (in the range above 550 nm) and thus provide a clear advantage regarding sample visibility, handling and prevention of contamination.



## Introduction

Numerous types of light-sensitive reagents and samples are often used in the laboratory routine. Once exposed to light their life-time or activity may rapidly decrease and therefore adversely affect subsequent assays and experiments. To avoid those difficulties light sensitive reagents and samples must be handled and stored in a manner that protects them from direct light exposure. Commonly used amber-colored laboratory plastic tubes in various volume formats reduce or completely block light transmission, particularly in the

energy-rich, short wavelength UV/VIS spectrum, and thus allow safe storage and handling of light-sensitive samples. While providing good sample protection, the majority of these tubes come with a major drawback: they are opaque and therefore do not allow direct sample visibility. In this Application Note we investigated both sample light protection (fluorescein recovery assay) and sample visibility (transmission spectrum) of several amber conical tubes.

## Materials and Methods

### Sample photoprotection: fluorescein recovery measurement

A light-sensitive fluorescein solution (excitation maximum: 495 nm) was used to evaluate the ability of tubes to protect light sensitive samples, when exposed to daylight for 92 hours. The 50 mL amber conical tubes of various manufacturers (Eppendorf, Gr, V, Ar, Lc) were filled with 30 mL of a 30 nM fluorescein solution diluted in 0.1 M NaOH. The standard (clear) Eppendorf 50 mL Conical Tubes were used as a negative control (no light protection) and standard (clear) Eppendorf 50 mL Conical Tubes wrapped in aluminum foil were used as the positive control (maximal light protection). The tubes were closed and incubated at room temperature at daylight (average daylight and lamp light conditions in the laboratory) or in the dark for 92 hours. After incubation, 190 µL of the fluorescein solution stored in each tube were used for fluorescence measurements in the Fluoroskan Ascent (Thermo Scientific). Each tube was tested in triplicate and two independent experiments were performed.

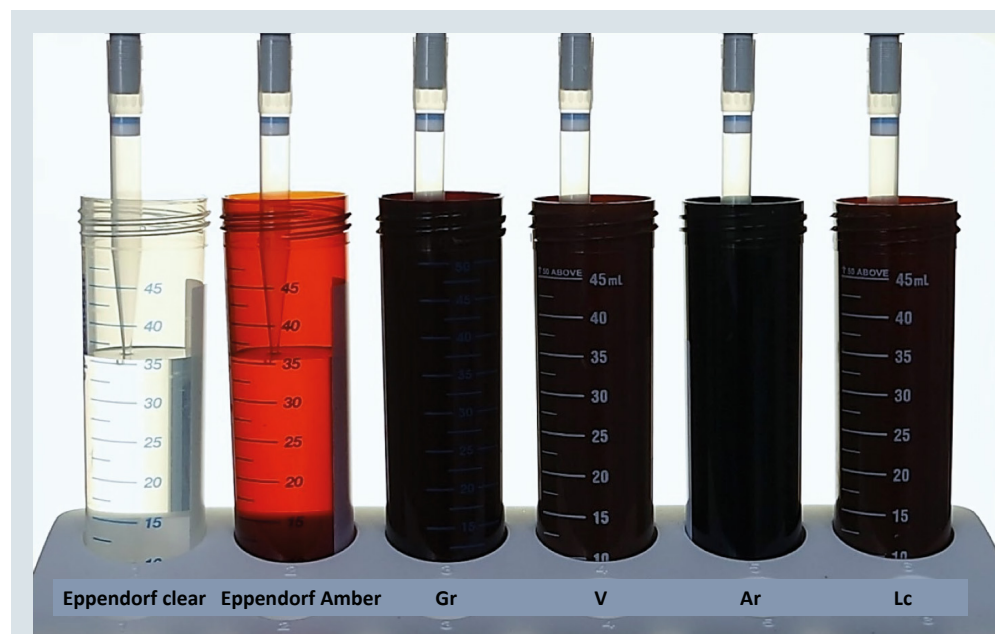
### Sample visibility: transmission spectrum measurement

A transmission spectrum was measured by using a method adapted from USP 660 and Eur. Ph. 3.2.1, from the chapter "Spectral Transmission for Colored Glass Containers" [1]. Two sections of approximately 5 x 5 mm were cut from the wall of each 50 mL amber conical tube: Eppendorf, Gr, V, Ar and Lc. The section was washed with water, dried and wiped with lens tissue. It was then placed in a holder allowing absorbance measurements in the Eppendorf BioSpectrometer®. An absorbance spectrum covering the range between 200 nm and 650 nm was generated for each wall section, which corresponds to the spectral range that includes the absorbance maxima of most dyes/chromophores employed in life science applications. The reference/blank sample consisted of a measurement without a section (air). The absorbance data (A) were converted into percentage transmittance data (T) based on the following equation:  $A = 2 - \log_{10} \% T$ .

## Results and Discussion

The amber conical tubes from different manufacturers show significant differences in terms of color intensity and opacity. As shown in figure 1 the color range varies from medium brown to black and transparency/sample visibility is either close to zero or zero in most cases. Eppendorf Amber Conical Tubes show significantly less coloring intensity as compared

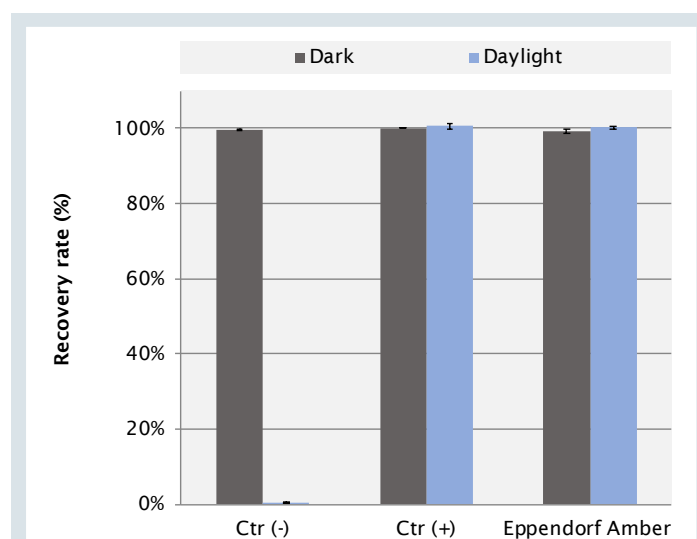
to the other tubes and they are the only tubes that are transparent. This represents a major advantage in terms of sample handling. It enables users to fully control the insertion depths of pipette tips and accurately see the liquid levels as well as avoid pipette contamination.



**Figure 1:** Visibility of samples and pipetting in various amber conical tubes.

The recovery rates of fluorescein samples (sample photo-protection) after 92 hours of incubation in Eppendorf 50 mL Conical Tubes (clear and amber) are presented in figure 2. As compared to the clear variant the Eppendorf amber tubes show very high recovery rates of fluorescein samples and thus provide nearly 100% protection. Recovery rates of fluorescein samples incubated in other amber tubes were similar (data not shown).

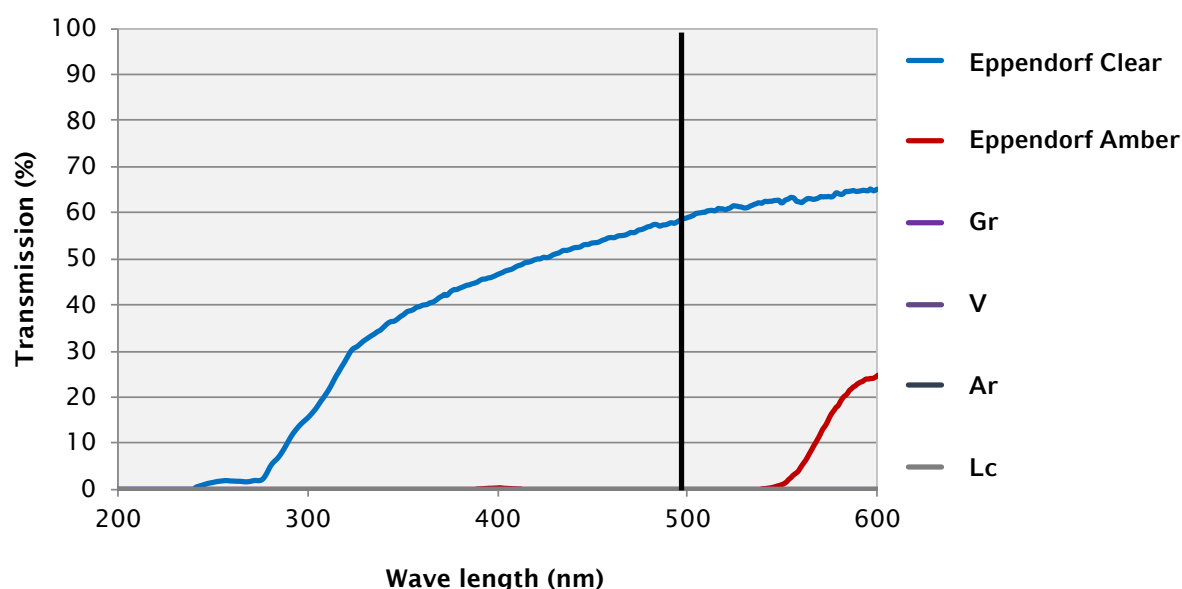
The analysis of the controls confirms that fluorescein is sufficiently sensitive to light (negative control: low recovery rates in clear tubes incubated under daylight conditions), whereas no unspecific degradation of fluorescein could be observed (positive control: normal recovery rates in tubes covered with aluminum foil). To further evaluate possible leaching of other fluorescently active substances from the amber tubes, which might also influence and bias the measurements, an additional control experiment was performed: after 92 hours of incubation of only the fluorescein dilution solution (0.1 M NaOH), no increase in fluorescence was detected for samples incubated in any of the tubes tested (data not shown).



**Figure 2:** Fluorescein recovery rate (percent) after 92 hours of incubation in 50 mL amber conical tubes at daylight or in the dark. Ctr (-): Eppendorf 50 mL Conical Tubes clear; Ctr (+): Eppendorf 50 mL Conical Tubes clear wrapped in aluminum foil

In figure 3 the transmission spectra (percentage of light transmission at different wave lengths) of amber conical tubes are compared. The good photo-protection properties of all tested amber conical tubes correlate with the light transmission percentage that is close to 0% when measured in the range between 200 nm and approximately 550 nm. This constitutes the active range of the majority of dyes and fluorophores used in the life science and other applications.

Above 550 nm the Eppendorf Amber Conical Tubes show a moderate increase in transmission, which allows good sample visibility and which correlates with the light brown coloring of the tubes. This transmission spectrum is similar to that of amber glass vessels used routinely in life-science, pharma and chemical laboratories and follows respective limits of spectral transmission set as standard in this field [2].



**Figure 3:** Transmission spectra (transmission percentage as a function of wave length) of amber conical tubes. The fluorescein excitation ( $\lambda_{ex}$ ) maximum is depicted as a solid black line.

## Conclusions

All 50 mL amber tubes tested in this Application Note provide good protection of light sensitive samples as indicated by high (nearly 100%) recovery rates of fluorescein samples following 92 h of incubation under regular light conditions. This result is also confirmed by the very low (close to 0%) light transmission in the energy-rich range of wave lengths

between 200 nm and approximately 550 nm. The Eppendorf Amber Conical Tubes are the only tubes that are transparent in the range above 550 nm and thus provide a clear advantage regarding sample visibility, handling and prevention of contamination.

## Literature

[1] Glass Containers for Pharmaceutical Use (3.2.1) p. 363; European Pharmacopeia 7.0; 01/2008

[2] Spectral Transmission for Colored Glass Containers; 4 (660) Containers—Glass / Physical Tests; USP Guidelines 36

### Ordering information

| Description                                                                                                                                                      | Order no.<br>International | Order no.<br>North America |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|----------------------------|
| <b>Eppendorf Conical Tubes 15 mL</b><br>sterile, pyrogen-, DNase, RNase and DNA-free, 500 tubes (10 bags x 50 tubes)                                             | 0030 122.151               | 0030122151                 |
| <b>Eppendorf Conical Tubes 50 mL</b><br>sterile, pyrogen-, DNase, RNase and DNA-free, 500 tubes (20 bags x 25 tubes)                                             | 0030 122.178               | 0030122178                 |
| <b>Eppendorf Conical Tubes 15 mL</b><br>sterile, pyrogen-, DNase-, RNase-, human and bacterial DNA-free, amber (light protection), 200 Tubes (8 bags x 25 tubes) | 0030 122.194               | 0030122194                 |
| <b>Eppendorf Conical Tubes 50 mL</b><br>sterile, pyrogen-, DNase-, RNase-, human and bacterial DNA-free, amber (light protection), 200 Tubes (8 bags x 25 tubes) | 0030 122.224               | 0030122224                 |

**Your local distributor:** [www.eppendorf.com/contact](http://www.eppendorf.com/contact)

Eppendorf AG · Barkhausenweg 1 · 22339 Hamburg · Germany  
[eppendorf@eppendorf.com](mailto:eppendorf@eppendorf.com) · [www.eppendorf.com](http://www.eppendorf.com)

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