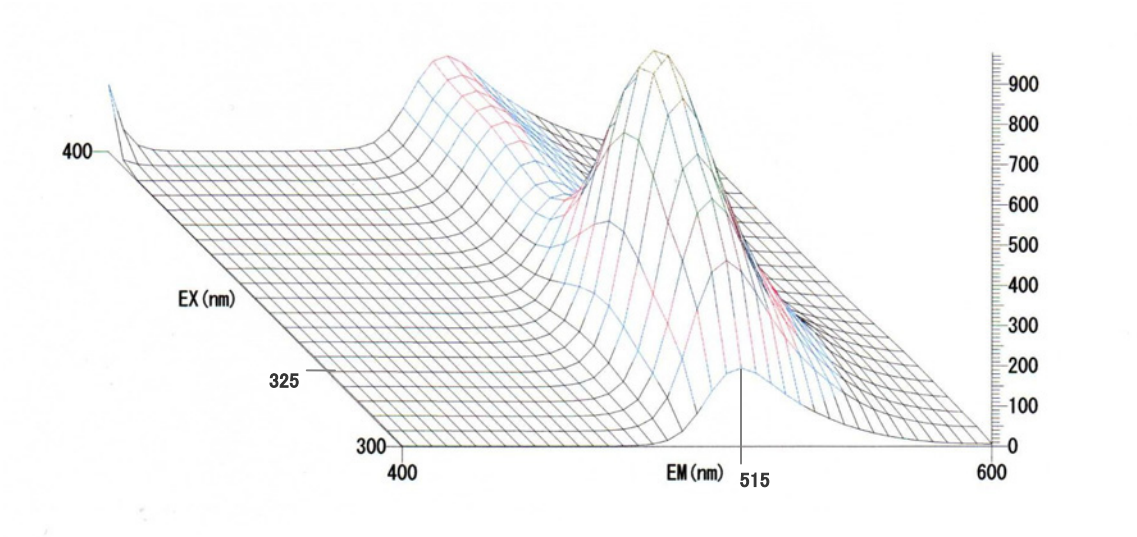
UV irradiation requirements for Loopamp® Fluorescent Detection Reagent

Visual fluorescence detection with Loopamp Fluorescent Detection Reagent can be conducted by UV irradiators such as transilluminator or black-light lamp. Due to the characteristics of Calcein(a fluorescent substance), be aware of the following information for handling:

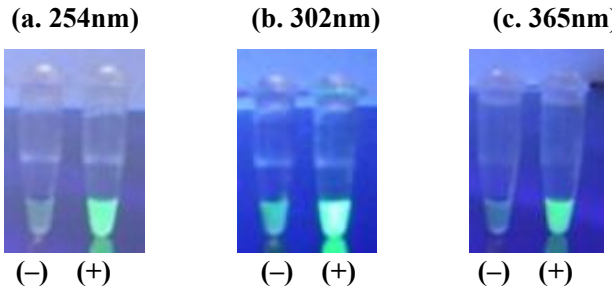
When Calcein is under the ultraviolet exciting light widely ranged from short wavelength (240nm) to long wavelength (370nm), the yellow-green fluorescence (about 515nm) was stimulated. Particularly for the exciting light around middle wavelength (325nm), the strongest fluorescence was observed. (Fig.1)

Fig.1 Excitation wavelength (EX) on Calcein Vs. emission wavelength (EM)



As a result, when the exciting light is near the middle wavelength (320nm), along with the strongest fluorescence observed from positive samples, the fluorescence background is also strengthened. (Fig.2-a,b,c)

Fig.2 Fluorescence of positive (+) and negative (–) samples under different excitation wavelength



Therefore, use exciting light with the short wavelength (240-260nm) or long wavelength (350-370nm) for visual fluorescence detection with Loopamp Fluorescent Detection Reagent. When UV lamp of wavelength around 320nm is used, negative sample may look like radiating fluorescence. Judgment should be done by comparing fluorescence of sample with that of positive and negative controls.

When output of UV lamp is too strong, negative control may look like radiating fluorescence. In such a case, take the UV lamp away from the reaction tube or change the angle of the reaction tube so that the difference between positive and negative controls becomes observable.