

Cuvette Coverslip Holders*, Model CCH-1 and CCH-2

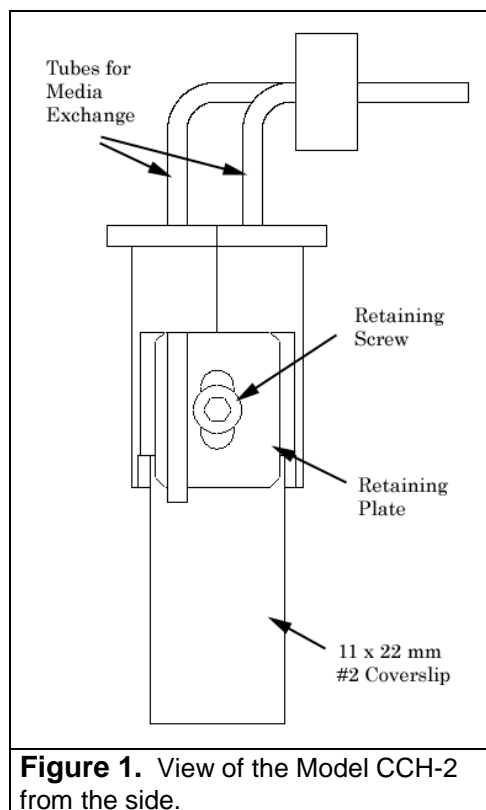


Figure 1. View of the Model CCH-2 from the side.

The model CCH-1 and CCH-2 are two plastic holders designed to properly hold a microscope coverslip in a standard 10 X 10 mm cuvette. The coverslip is held at 45° relative to the excitation beam. It can be used to monitor the fluorescence of cells attached to the coverslip. The holder is designed to firmly hold #2 size coverslips having the dimensions of 11 X 22 mm. This style of coverslip is available from Thomas Scientific (part no. 6663-Q10). All wetted parts of the holder are made from either stainless steel or PET (polyethylene terephthalate). The tubing used to conduct fluids to and from the holder in the Model CCH-2 is made of polyurethane.

The Model CCH-2 holder contains two tubes that can be used to exchange fluids in the cuvette. This is typically used for perfusion experiments, allowing the cell media to be changed during the course of the experiment. The Model CCH-1 is identical to the CCH-2, except that it does not contain these tubes. Both models contain a small access hole on the top of the holder. The diameter of the hole is 0.031 inches (0.8 mm). It is designed for injection of reagents into the cuvette during the course of an experiment. This can be accomplished using a microliter syringe (Hamilton #702, or equivalent). The position of this hole in the top of the holder is conveniently aligned

with a similar hole in the lid of the C&L Instruments Cuvette Accessory, Model CV-1.

Instructions for Use

Figure 1 illustrates the cuvette holder as viewed from the position in which the coverslip is mounted. To mount the coverslip, loosen the *Retaining Screw* and *Retaining Plate*, slip the coverslip under the *Retaining Plate* and carefully tighten the *Retaining Screw*. The *Retaining Screw* uses a 5/64 inch Allen key. The coverslip is normally mounted with the cell surface facing up while viewing the holder as shown in Figure 1.

In order to avoid excessive background signal in the fluorescence readings, it is advisable that the coverslip holder not be placed in the cuvette in a position which would cause the cover slip to reflect the excitation light directly into the light path of the fluorescence emission. Figure 2 illustrates the preferred orientation of the coverslip holder with respect to the excitation and emission optics. If the coverslip is mounted such that the cell layer is on the top surface, as in

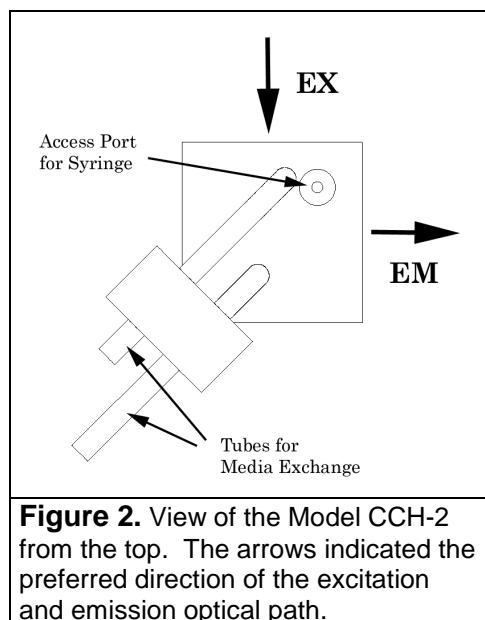


Figure 1, orientation of the holder as in Figure 2 will allow the excitation beam to excite the cells without having to pass through the glass coverslip. The emission will then be measured after having passed through the coverslip. Scattered excitation light reflected off the surface of the coverslip will not be directed into the emission detector.

Mixing and Changing Media

The coverslip holders have been designed for use in the cell chambers of fluorometers that have a Z-height of 15 mm. The Z-height is the distance from the bottom-most surface of the cuvette to the center of the optical path. The center of illumination is approximately in the center of the coverslip when mounted in the holder.

The holder places the bottom of the coverslip at a height above the bottom of the cuvette so that a small magnetic stir bar can be placed in the cuvette for mixing purposes

without the stir bar interfering with the coverslip. Two small stainless steel tubes are provided in the model CCH-2 to allow the exchange of the media in the cuvette. The stainless tubes have an OD of 1/16 inch. These tubes connect to polyurethane tubing. The polyurethane tubing has the dimensions of 1/16 inch ID and 1/8 inch OD. To use this feature, connect one of the tubes to a small infusion pump and connect the other tubing to an air vacuum system. Since the top of the coverslip holder does not seal to the cuvette in a watertight fashion, excess media must be removed by an aspiration vacuum to prevent the media from overflowing the contents of the cuvette. The rate of media exchange can be controlled by the rate of the infusion pump.

If the contents of the cuvette are well mixed, the time it takes to exchange the media can be calculated from the following equation.

$$Conc_t = Conc_0 \left(1 - e^{-\left(\frac{rate}{volume}\right)time} \right)$$

Where:

Conc_t = the concentration of a metabolite at time t

Conc₀ = the concentration at time zero

Rate = the rate of infusion by the infusion pump

Volume = the size of the mixed volume in the cuvette.

For instance, it will take 7.5 minutes to exchange 95% of the media using an infusion rate of 0.8 ml/min, assuming a mixed cuvette volume of 2.0 ml.

*Patent Pending

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