



IMS In Vitro SPF/UVA Protocol for use with *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] Substrate

Part One: Setting up a Hydration Chamber

Background

The quantity of water absorbed by human stratum corneum during product application, rub-out and dry-down impacts on how various emulsions spread and de-emulsify on skin. Our research indicates that water absorption by substrate (either human skin or *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®]) can have a significant effect on measured SPF and UVA protection factors. Dramatic improvements in the correlation with *in vivo* testing can be achieved by reproducibly hydrating the substrate prior to product application. *(Please Note: VITRO-SKIN*[®] *or VITRO-CORNEUM*[®] *has a shelf life of approximately 8 months if stored in a cool, dry, dark environment. It does not need to be refrigerated).*

Hydration Chamber Instructions

Use an IMS HYDRATION CHAMBER or locate an unused desiccator with an internal shelf. Remove the shelf(s) and any desiccant and then thoroughly wash and dry all equipment. Add 298 grams of water to a 500 ml beaker. Add 52 grams of glycerin and mix well (350g total – this amount is used with the Red Lid Hydration Chamber – 2.5gallon). Pour the glycerin/water solution into the bottom of the hydration chamber. Place the shelf(s) in the hydration chamber and replace the lid (making sure that it achieves a good seal). Be careful when removing the lid of the chamber so it does not tip and splash fluid on the hydrating *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®]. When exposed to constant temperature this system will maintain a controlled humidity environment for hydration of *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®]. Our hydration studies indicate that a controlled temperature of 23 °C yields optimal results. *Note: this glycerin/water mixture is unpreserved--- and to prevent the growth of mold, it should be discarded after 3 days. In addition, the hydration chamber and shelve(s) should be sanitized each time the glycerin/water solution is changed.*

Part Two: Substrate Preparation

Carefully remove the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] from its protective packaging. Cut the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] into 6.2 cm x 9.0 cm rectangles using a high-quality paper cutter or shears (see figure 1). Place the film in a closed, controlled-humidity chamber (see Part One) for **16-24 or 6-8 hours** respectfully prior to product application. *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] should not be left in the hydration chamber for more than 24 hours. The humidity in the chamber is regulated by a solution of 85% water / 15% glycerin, placed in the bottom of the chamber. *The substrate is placed above the liquid on a shelf or tray.* This step insures reproducible hydration of the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] prior to product application.

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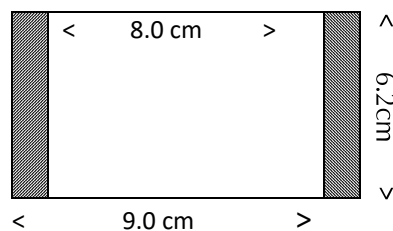
Part Three: Product Application

Turn on the spectrophotometer and follow the manufacturer's directions for start up and calibration.

It is recommended that (1) the reference pieces and the sample pieces used in the following steps be from the same sheet of *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] and (2) the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] should be properly hydrated (as outlined in Part Two).

1. Mount a small piece of *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] in a 35 mm slide mount with the "skin topography" side up (the dull or non-shiny side). This will be used as a **reference** for the in vitro SPF measurement.
2. Carefully draw 100 µl of the test sample into a calibrated, positive-displacement pipettor. Dispense the product onto a paper towel and check for the presence of air bubbles. If no air bubbles are observed, carefully refill the pipettor with product.
3. Remove a 6.2 cm x 9.0 cm piece of hydrated *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] from the hydration chamber and place it on the plastic-covered foam block from the IMS *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] Starter Kit (used to simulate the flexibility of the human dermis). Product application **must** be made to the "skin topography" side of the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] (the dull or non-shiny side). Pipette the 100 µl of product evenly across a **6.2 cm x 8.0 cm** section of the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] (Figure 1) by dotting it at approximately 30 equally spaced points across the substrate (this results in a product dose of 2 µl/cm²). Use the outer 0.5 cm (shaded areas in Figure 1) to hold the substrate while applying the product (*no product should be spread over the shaded areas in Figure 1*).

Figure 1



4. Immediately after product application, rub the product into the film with a gloved finger. During the rub time, press with sufficient force to temporarily deform the plastic-covered flexible foam block. Rub the product into the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] as you would on human skin *in vivo*. Specifically:
 - use a similar amount of force,
 - initially rub the product into the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] with a circular motion,
 - finish with a "back & forth" motion.
5. Trim the film with scissors or just snap it into the 6 cm x 6 cm glassless slide mount.

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6. Start a timer (to time the 15-minute dry-down time). Make UV transmission measurements (Part Four) on this slide immediately after the 15-minute dry-down. Our research has shown that in order to get the best possible, reproducible results, the humidity and temperature should be kept as constant as possible. We recommend that the humidity is between 50% and 60% at a temperature of 70 degrees Fahrenheit. This will eliminate seasonal differences (dry in the winter, and humid in the summer) which definitely affect the dry down of emulsions.
7. Prepare slide two (repeat steps 2 thru 6).

Part Four: Making the UV Forward Scattering Measurements

1. The spectrophotometer should already be on (and warmed up if necessary). If not, follow the manufacturer's directions for start up prior to making the initial measurement.
2. Run a reference scan on the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] reference mounted in the 35 mm glassless slide mount. (Recall that this was prepared in Part Three, Step 1.) This reference piece of *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] **should be from the same sheet of film that was used for the product, and must have been pre-hydrated in a similar fashion.** The "skin topography" side of the *VITRO-SKIN*[®] or *VITRO-CORNEUM*[®] (the dull or non-shiny side) should be up.
3. Place product **slide one** above the integrating sphere. Make a minimum of five consecutive measurements on five separate areas of the slide.

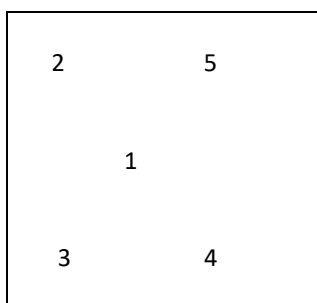


Figure 2

4. ***(For Optometrists SPF-290 users only)*** Repeat the reference scan (step 2) to generate a reference scan for the product on **slide two**. *(Please note: This step is not required when using a Lab sphere UV-1000 or other spectrophotometers that do not require a second reference scan).*
5. Repeat step 3 for product **slide two** (a minimum of 5 separate measurements).

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