

**APPENDIX 2**

## **Appendix 2: Protocol For Critical Wavelength Determination**

### **Substrate Preparation:**

Synthetic collagen substrate with simulated skin topography on one side (Vitro-Skin - Innovative Measurement Solutions Inc., Milford, CT) was placed in a room temperature ( $22 \pm 2$  °C) 80-90% relative humidity chamber, topography side up, for  $\geq 24$  hours to hydrate. After the collagen was hydrated, it was cut into 9 x 10.2 cm rectangles and returned to the humidity chambers, still topography side up.

### **Product Application:**

For each product run,  $1 \text{ mg/cm}^2$  (91.8 mg) of product was evenly applied to the topography side of a piece of the hydrated collagen with a finger covered with a latex finger cot. The product film was then allowed to dry under ambient conditions ( $22 \pm 2$  °C) for 15 minutes.

### **Pre-Irradiation:**

After the dry down period, samples were then irradiated with broad band UV radiation from a Oriel 1000 W Xenon Arc Solar Simulator (Oriel Corp., Stratford, CT) whose output was filtered (dichroic mirror, Oriel filters 81017 and 81018) to simulate the solar ultraviolet spectrum. The average UV power output (beam intensity per run) from this solar simulator to the product films was  $6.9 \text{ mW/cm}^2$ , as measured using a spectroradiometer (model 754, Optronics Co., Orlando, Fla.

For this pre-irradiation, the UV fluence used was equal to two thirds of the labeled (commercial products) or clinically measured (prototype sunscreen formulations) product SPF, in  $\text{J/cm}^2$ .

### **Absorbance Measurements:**

Immediately after UV pre-irradiation, the UV absorbance of the product film was measured using a Labsphere UV-1000S Ultraviolet Transmittance Analyzer (Labsphere Inc., North Sutton, NH). This was accomplished by first taking a baseline absorbance measurement at eight different sites of a 9 x 10.2 cm rectangular piece of untreated, hydrated Vitro-Skin using the Labsphere UV-1000S. Next, the UV absorbance of the product film was measured in eight different sites. Finally, Critical Wavelength and other UV parameters were calculated and printed by the Labsphere instrument using known relationships between absorbance, transmittance, and SPF, as well as well known numerical integration techniques (Trapezoidal Rule).

### **Number Of Replicates:**

For each product sample, a total of five independent replicate product films were prepared and evaluated.

### **Critical Wavelength Determination:**

The ninety-five percent (95%) lower confidence limit computed from the 5 individual replicates was used as the Critical Wavelength determination for each product.