Title: Procedure to Extract Urine Samples for the 8-Isoprostane EIA

Purpose: To assist the laboratorian in utilizing the Cayman Chemical 8-Isoprostane 4mL Affinity Columns to purify urine samples for the 8-Isoprostane EIA

Procedure:
1. Assemble the supplies and reagents required for the extraction:
   a. 8-Isoprostane Affinity Columns, 4mL (Cayman Chemical, catalog #416358), one per sample
   b. Column support racks
   c. Eicosanoid Affinity Column Buffer (5X) (Cayman Chemical, catalog #400220), dilute the 250mL bottle to 1250mL with ultrapure water
   d. Eicosanoid Affinity Column Elution Solution (Cayman Chemical, catalog #400230), 95% ethanol, 5% ultrapure water
   e. 12 X 75 mm test tubes labeled with the pick numbers of the samples to be extracted. Be sure the labels are well affixed to the tubes as they will be stored at -80deg C.
   f. Ultrapure water, 12 X 75 test tubes, tube caps, pipets
2. Thaw, mix well then centrifuge the urine samples to be extracted
3. Remove the 8-Isoprostane Affinity Columns from the refrigerator. Number the columns to correspond with the pick numbers of the samples to be extracted. Remove the upper cap of each column first before removing the lower cap or tab to prevent bubbles from forming in the sorbent packing material. Be sure the buffer is flowing through the column before placing it in the column rack over a 12 X 75 mm test tube. If necessary, place a gloved finger over the top of the column and press gently to initiate the flow.
4. Once the columns have completely drained, pipet the clear upper portion of the urine samples onto the columns, being sure to match each sample pick number with the matching number on the column. Use each column only once with a maximum of 1mL of urine.
5. Once the samples have been entirely absorbed onto the column packings, pipet 2mL of Eicosanoid Affinity Column Buffer into the reservoir of each column. Note that the collection tubes will need to be emptied.
6. When the buffer has passed completely through the columns, pipet 2mL of ultrapure water into the reservoir of each column.
7. After the columns have completely drained, move the columns over the 12 X 75 mm tubes with numbered labels, being careful to match the numbers on the columns with the numbers on the tubes. Pipet 2 mL of Eicosanoid Affinity Column Elution Solution onto each column.
8. When all the Elution Solution/sample is collected, cap the tubes, snap-chill the samples in liquid N2, and place the sample eluates upright in a -80deg C freezer for storage up to one year.