

Soil Extraction Procedure for Dioxin

Extraction Procedure

1. Weigh 5 gm of soil into a 30 mL HDPE bottle.
2. Add 6 gm of anhydrous sodium sulfate and mix until sample is free flowing.
3. Add 1 steel mixing ball.
4. Add 20 mL of 20% acetone in hexane. Shake for 1 hour.
5. Allow to settle for 1 hour or centrifuge for 5 min. at 3000g.
6. Transfer extract to a 40 mL screw cap vial.

Sulfuric Acid Treatment

1. Add 8 mL of concentrated sulfuric acid to vial containing extract.
2. Shake by hand for 2 min. (Be sure to release pressure before shaking.)
3. Allow phases to separate and transfer the extract (top layer) to a clean 40 mL vial.
4. Transfer 5 mL of the extract to a 7 mL test tube and evaporate to 1 mL with nitrogen. This 1 mL will be applied to the multilayer silica column.

Multilayer Silica Column Clean Up

Multilayer Silica Column Preparation

1. Activate Silica Gel – Wash 60-100 mesh silica gel with methylene chloride. Allow the methylene chloride to evaporate under a fume hood with frequent stirring of the silica gel. When all of the methylene chloride has evaporated, bake the silica gel at 180°C for at least 1 hour. Cool in a desiccator. Store in a pre-cleaned glass jar at room temperature in a desiccator.
2. Prepare 44% Sulfuric Acid Silica Gel – In a clean glass jar; add 4.28 mL of sulfuric acid to 10 gm of activated silica gel. Stir and shake until a uniform mixture without any lumps is obtained. Cap and store in a desiccator at room temperature for at least 24 hours prior to use.
3. Prepare Basic (2% NaOH) Silica Gel – In a clean glass jar, add 4.85 mL of 1N sodium hydroxide to 10 gm of activated silica gel. Stir and shake until a uniform mixture without any lumps is obtained. Cap and store in a desiccator at room temperature for at least 24 hours prior to use.
4. Prepare Multilayer Silica Column – Obtain a 20 mL filtration column. Place a frit in the bottom. Add the following layers: 0.5 gm activated silica, 1 gm basic silica, 0.5 gm activated silica, 2 gm sulfuric acid silica gel, 0.5 gm activated silica, 1 gm sodium sulfate. Place a frit over the top layer (sodium sulfate). Store in desiccator at room temperature until use.

Extract Clean Up Using Multilayer Silica Column

1. Pre-elute multilayer silica column with 30 mL of hexane.
2. Add the 1 mL of extract from the sulfuric acid treatment step to the column. Rinse the test tube with 2-1 mL portions of hexane and apply to the column. Discard the solvent.
3. Elute the column with 30 mL of hexane and collect in a 30 mL or larger test tube.
4. Evaporate with nitrogen to 4 mL or less.
5. Transfer to a 7 mL test tube. Rinse the 30 mL test tube with 3-1 mL portions of hexane and add to the 7 mL tube.
6. Evaporate with nitrogen to 0.5 mL or less (do not completely evaporate to dryness). Add 100 µL of DMSO with 0.02% Triton X-100 (DMSOT).
7. Evaporate all of the hexane so only DMSOT is left. It is very important that no hexane is left. It will interfere with the ELISA.

8. Add 400 μL of DMSO. Add 500 μL of deionized water to bring to 1 mL. Transfer to a 4 mL vial with a Teflon lined lid.
9. Dilute 1:10 with 50%DMSO in deionized water with 0.01% Triton X-100. Analyze in ELISA.

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