



Micro-Tracers Inc.

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Quality Assurance With Microtracers RF-Lake

Principles:

Microtracers® are easily identifiable "harmless markers" used to assure the quality of mixed formula animal and poultry feeds. When formulated in vitamin, mineral or medicated premixes, a Microtracer serves to mark the presence of the premix in the finished feeds. When assayed quantitatively, Microtracers can be used to document efficacy of mixing as well as adequacy of batch to batch "cleanout" of mixers and other feed manufacturing equipment (this does not apply to RF tracers). Microtracers are isolated from feed or premix samples either utilizing a Mason Jar with a magnetic lid or a Microtracer Rotary Detector™ magnetic separator. With the Rotary Detector samples as large as 500 grams may be assayed routinely.

Microtracer RF-Lakes designed to be used in intermediate- or high-moisture feeds or premixes with maximum 50% moisture. Microtracer RF-Lakes may be assayed qualitatively using the Mason Jar technique or a Rotary Detector technique (two minutes) or quantitatively using a Rotary Detector or "magnetic wand" to isolate the tracer from the feed, and then detection by spectrophotometer (five to ten minutes). Lake colors are easily differentiable from our standard F, FS or RF tracers formulated with water soluble dye because little color will elute when using water or alcohol to develop the color. In addition to qualitative analysis, Microtracer RF-Lake may be assayed semi-quantitatively by eluting the dye from the tracer, reading its absorbance at a specific wavelength, and comparing it to a set of standard curves.

Specification:

Microtracers RF-Lake consist of fine hydrogen reduced elemental iron particles (100% passing 40 mesh, USA Standard Sieves), one or more artificial "lake" colors, and a food-grade water-insoluble coating agent. Colors include Blue, Red, Orange, Yellow, Green, and various combinations. Microtracers RF-Lake are not intended to be counted as the particles are too small to yield clearly distinct spots.

Microtracers RF-Lake are designed to avoid loss to magnetic separators in feed mills with less than 1% typically lost to such magnets.

Tracer recovery from feeds typically averages 100% from a mixer to which they have been directly added, 90% from finished mash feed at loadout and 70% for pelleted feed at loadout (these recoveries assume use of a Rotary Detector with special "rare earth" magnet or a special "rare earth magnetic probe" for magnetic separation of the tracer from feed samples).

Applications and Amount of Use:

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1. Routine Identification of Premix in Finished Feeds

Premixes should be formulated to yield between 2 and 5 grams of tracer per ton of finished feed if a Rotary Detector will be used for the analysis and minimum 300 gram sample analyzed. Premixes must be formulated to yield 5 grams of tracer per ton of final feed if a Mason Jar test procedure will be used. If a premix is added to the feed at 500 g per ton, then 2 grams of tracer minimum should be formulated per kilogram of premix.

For greater confidence and to measure "carryover" of premixes coded with Microtracers RF-Lake, one should use a Rotary Detector™ to test for microtracers. This permits complete tracer recovery and analysis of larger feed samples (i.e. 500 grams). The chances of obtaining a "false negative" (coded premix present at formulated level but no tracer found) will be nil. The likelihood of finding some tracer particles if 10% "carryover" of the premix to a non-target feed occurs will be greater than 95% if the tracer is formulated to yield 5 grams per ton of final feed.

2. Product Identification

Microtracers RF-Lakes may be formulated to code premixes and feeds containing them as proprietary. This is useful in protecting patent or distribution rights, in servicing improper product liability claims or requests for services and in controlling use of proprietary feed (i.e. misuse of feed by contract growers).

Detection Procedure - Mason Jar Technique (Qualitative)

Materials:

- a. A scale suitable for weighing approximately 100 grams of feed.
- b. Whatman #1 filter paper, 7.0 cm circles.
- c. For pelleted feeds, a coffee mill or osterizer.
- d. A dropper bottle or transfer pipette.
- e. 7 % sodium carbonate solution.
- f. A Mason Jar with a magnetic lid (supplied by Microtracers, Inc.).
- g. A mug warmer or hot plate.

Procedure:

1. Prepare pelleted feeds for analysis by grinding them to mash.
2. Transfer 100 grams of feed to Mason Jar.
3. Insert one sheet of filter paper into special magnetic lid and screw lid onto Mason Jar.

4. Shake the jar for one minute, exposing all feed to the magnetic lid.
5. Remove the lid, placing it upside down with filter paper fully exposed.
6. Place five to ten drops of developer (7% Sodium Carbonate in water) in the center of the exposed filter paper so the developer diffuses through the ring of RF-Lake particles on the filter paper.
7. Transfer the paper to a mug warmer or hot plate and dry it. color will develop as the paper dries.

Total Elapsed Time: Less than 2 minutes

Detection Procedure - Rotary Detector Technique (Qualitative):

(Retrieval from mash or pelleted feeds)

Materials:

- a. Coffee mill, or equivalent, for grinding pelleted samples to a fine consistency
- b. Rotary Detector magnetic separator with “rare earth” magnetic platform or special “rare earth” magnetic probe, for isolating the tracer from premixes or ground feed samples.
- c. Scale

Procedure:

Weigh 500 g to 1.0 kg coarse or pelleted samples and grind in the coffee mill. Pour the finely ground feed through the Rotary Detector two times to isolate very nearly 100% of the retrievable iron from the feed. The tracer is now prepared to be developed.

Development:

1. Carefully lift the filter paper with the retrieved RF-Lake particles from the magnet in the Rotary Detector and place it on a flat surface.
2. With an eye-dropper or spray bottle, thoroughly wet the filter paper with the developer (7% Sodium Carbonate in water). Wait for a few seconds for the developer to penetrate the filter paper. A ring of tiny colored spots will appear.
3. Transfer the wetted filter paper to a hot plate pre-heated to no more than 100°C or to a pre-heated oven. Dry the paper.
4. When the paper is dry, the color of the ring will indicate the nature the tracer.

Detection Procedure - Rotary Detector Technique (Quantitative):

This procedure is written for determination of dye content in **Microtracer RF -Blue #1 lake**. It can be used for determination of dye content in F-, FS- RF-Microtracers, containing other Lake dyes. However, the position of wavelengths for this determination should be taken from following table.

Dye:	Common Name:	Wavelength Maxima:
Red #3	Erythrosine	525nm
Red #2	Amaranth	520nm
Red #40	Allura Red	510nm
Blue #1	Brilliant Blue	630nm
Blue #2	Indigo Blue	610nm
Patent Blue		630nm
Yellow #6	Sunset Yellow	482nm
Yellow #5	Tartrazine	425nm
Green #3	Fast Green	620nm
Green Supra		634nm

Materials:

- a. Coffee mill, or equivalent, for grinding pelleted samples to a fine consistency
- b. Rotary Detector magnetic separator with “rare earth” magnetic platform or special “rare earth” magnetic probe, for isolating the tracer from premixes or ground feed samples.
- c. Scale
- d. Spectrophotometer

Procedure:

Weigh 500 g to 1.0 kg coarse or pelleted samples and grind in the coffee mill. Pour the finely ground feed through the Rotary Detector two times to isolate very nearly 100% of the retrievable iron from the feed. The tracer is now prepared to be developed.

Development:

1. Carefully lift the filter paper with the retrieved RF-Lake particles from the magnet in the Rotary Detector and place it on a flat surface.
2. Place ~25 milligrams of FD&C Blue #1 lake dye **STANDARD** in 100 ml volumetric flask and bring to volume with 1% sodium carbonate aqueous solution* and agitate for at least 40 minutes by a magnetic stirring bar to dissolve the lake dye. Usually the purity of lake dyes range from 38% to 42%. You need to know this number to calculate the % dye on tracer.
3. Place 1.00 to 1.50 grams of **RF-Blue #1 lake** in a 100 ml volumetric flask and bring to volume with 1% sodium carbonate aqueous solution and agitate for at least 40 minutes by a magnetic stirring bar to dissolve the dye on the tracer.

4. Dilute the lake dye standard solutions (1&2) 100 times with DI water (i.e., dilute 1 ml to 100 ml).
5. Dilute the sample solution (3) 40 times with DI water (i.e., dilute 5 ml to 200 ml).
6. Set the Spectronic 20 to 630 nm wavelength and blank the machine using D.I. water. Read the absorbance of each dilution for standard (4) and sample (5) for dye FD&C Blue #1.

(* - For all other lake dyes except Blue#1 Lake 7% sodium carbonate aqueous solution is recommended)

Calculation of Lake Dye Concentration:

$$\% \text{ Dye on Tracer} = 40 \times A (\text{sample}) / A (\text{standard}) \times Wt (\text{standard}) / Wt (\text{sample}) \times P (\text{lake dye})$$

A (sample)	Absorbance of sample dilution
Wt (standard)	Weight of lake dye standard
P (lake dye)	Purity of lake dye standard in decimal form
40	Dilution factor for sample
A (standard)	Absorbance of lake dye standard dilution
Wt (sample)	Weight of tracer sample

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