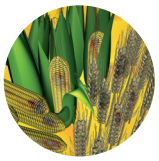

Application Note

RIDASCREEN® Aflatoxin M_1 (Art. No. R1121)

Butter

Sample Preparation

1. weigh 3 g butter into a 10 ml centrifugal vial with screw cap
2. centrifuge shortly (e.g.: 1 min/3000 g) at room temperature
3. melt the butter in a water bath at approximately 40 °C (104 °F)
4. add 3 ml n-hexane and mix
5. add 3 ml 70 % methanol
6. mix vigorously for 1 min on a vortex
7. continue mixing by rotating the vial end over end for 15 min
8. centrifuge 10 min/4000 g/10 °C (50 °F)
9. transfer an aliquot of the aqueous layer into a new vial[#)]
10. dilute 1:17 (1+16) with sample dilution buffer (see 4. Reagents provided, R1121)
    e.g.: 50 µl + 800 µl buffer
11. use 100 µl/well for testing

[#)] Recommendation:
With a variable pipette adjusted to 50 µl carefully pass the fat-hexane layer. Hold the tip in the middle of the aqueous layer, press some air bubbles out the tip and aspirate exactly 50 µl of the aqueous liquid. Wipe the outer side of the tip, in order to remove any fat residue.
Transfer the liquid into a new vial, add 800 µl buffer and mix.

Dilution factor: 20[*)]

[*)] Dilution factor 20 was established taking into consideration the water content of butter

Evaluation of the method

Spiking

- Add 150 µl Aflatoxin M1 spiking solution (10 ppb) onto 3 g butter → **500 ppt**
- Spike control:
  dilute spiking solution (10 ppb) 1:500 (1+499) with sample dilution buffer → **20 ppt**
  [1.dilute spiking solution (10 ppb) 1:50 (1+49) with sample dilution buffer,
  e.g.: 20 µl spiking solution + 980 µl buffer → 200 ppt;
  2. further dilute 1:10 (1+9), e.g.: 100 µl spiking solution, (200 ppt) + 900 µl buffer
  → 20 ppt]

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