

## MARIA® Assay Buffer Formulary (1 liter)

PBS pH 7.4, 0.02% Tween, 1% BSA

Reagent	Amount Required	
1X PBS (made with Type 1 ultrapure H <sub>2</sub> O)	1000 milliliters	
BSA, Fraction V heat shock ONLY (e.g. Roche 3116964001)*	10 grams	+/- 0.1 grams
Tween-20	0.2 milliliters	

\*If using this buffer with MARIA® for Foods milk analytes, we recommend testing BSA for Bos d 5 and Bos d 11 contamination prior to use.

### Method

1. Add approximately 800mL of 1X PBS to a 1L container and stir with a magnetic stir bar.
2. Trim the end of a 1000µL pipet tip (remove about 2mm) and use it to SLOWLY draw the Tween (Tween is viscous and care must be taken to measure the correct amount).
3. Slowly dispense the Tween into the buffer and then pipet several times to rinse the Tween from the tip.
4. Add the required amount of BSA and continue stirring to dissolve.
5. Transfer the buffer to a graduated cylinder and add 1X PBS to achieve the 1L final volume.
6. Transfer back to the transparent container and stir for at least 5 minutes.
7. Use a standardized pH meter to check the pH. The pH should be between 7.3 and 7.5.
8. If the pH is not within the acceptable range, consult with your QA Specialist.
9. If the pH is acceptable, pass the buffer through a 0.22µm membrane filter into a 1L bottle.
10. Label the bottle with the buffer name, preparation date, expiration date, and initials.
11. Store at 4°C for up to 2 weeks.

## PBS-T Buffer Formulary (1 liter)

PBS pH 7.4, 0.05% Tween

Reagent	Amount Required
1X PBS (made with Type 1 ultrapure H <sub>2</sub> O)	1000 milliliters
Tween-20	10 milliliters

### Method

1. Add approximately 800mL of 1X PBS to a 1L container and stir with a magnetic stir bar.
2. Using a 10mL pipet, SLOWLY draw the Tween (Tween is viscous and care must be taken to measure the correct amount).
3. Slowly dispense the Tween into the buffer and then pipet several times to rinse the Tween from the tip.
4. Transfer the buffer to a graduated cylinder and add 1X PBS to achieve the 1L final volume.
5. Transfer back to the transparent container and stir for at least 5 minutes.
6. Use a standardized pH meter to check the pH. The pH should be between 7.2 and 7.5.
7. If the pH is not within the acceptable range, consult with your QA Specialist.
8. If the pH is acceptable, pass the buffer through a 0.22µm membrane filter into a 1L bottle.
9. Label the bottle with the buffer name, preparation date, expiration date, and initials.
10. Store at room temperature for up to 4 weeks.
11. Use as ELISA assay wash buffer or as the extraction buffer for dust and air filter samples.



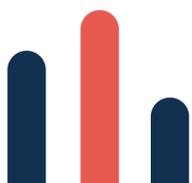
# Dust Extraction Protocol

## Method

1. Sieve dust through a US No. 18 mesh screen or higher (1mm opening or smaller) to remove large particles and fibers.\*
2. Weigh 100mg ( $\pm 5$ mg) fine dust into a 75mm x 12 mm plastic test tube (Sarstedt No. 55.476). If there is less than 100mg take a minimum of 10mg for extraction.
3. Add 2.0ml PBS-T (0.05% Tween 20 in phosphate buffered saline, pH 7.4) to a sample weighing 100mg (50mg/ml). For samples between 10mg and 100mg add the proportional amount needed. The amount of dust in mg is multiplied by 20 to give the appropriate volume of buffer in  $\mu$ l needed. Samples <10mg are labeled as "Not Enough Sample" and not processed without customer approval.
4. Resuspend using a vortex mixer (Vortex-Genie, Fisher Scientific).
5. Mix for 2 hours on a laboratory tube rocker at room temperature. Alternatively, a laboratory rotator with end over end mixing is sufficient.
6. Centrifuge 20 minutes at 300 x g and 4°C.
7. Remove supernatant (approximately 1.5ml) with a pipette, for measurement of antigen. Discard dust pellet.
8. Store extract (supernatant) at -20°C in a freezer vial with sample number or relevant code clearly labeled for future analysis of allergen content.

\*Whether to sieve the dust or not is determined by the quality of the dust. We do not routinely sieve dust collected from beds and soft furnishings. Carpet samples are sieved to separate the fiber and large particles from fine dust. We recommend the use of the DUSTREAM® which greatly reduces the need for sieving dust.

After collecting a dust sample using the DUSTREAM® dust collector, remove the nylon filter containing the dust sample, invert the filter and tap the dust onto a piece of weigh paper. The fine dust falls before the fibers come out of the filter. If fine dust does not fall out, use tweezers to remove the entire filter contents over a sieve with a piece of weigh paper underneath to catch fine dust.



# Apollo Air Filter Extraction Protocol

## Method

1. Place the filter back into a ziplock bag or a suitable container.
2. Add 10mls extraction buffer (e.g. PBS-Tween 0.05%)\* to the bag/container and seal.
3. Extract for 2 hours at room temperature with gentle agitation (laboratory rocker or shaking incubator).
4. Remove supernatant with a pipette and store extract for 1 day at 4°C or longer term at -20°C until allergen measurement can be performed.

*\*alternative extraction buffers can be used, contact InBio for any queries.*

# MARIA<sup>®</sup> for Foods Extraction Buffer Formulary (1 liter)

PBS pH 7.4, 2% Tween, 1M NaCl

Reagent	Amount Required	
1X PBS (made with Type 1 ultrapure H <sub>2</sub> O)	1000 milliliters	
Sodium Chloride - NaCl (ACS grade)	58.44 grams	+/- 0.1 grams
Tween-20	20 milliliters	

## Method

1. Add approximately 800mL of 1X PBS to a 1L container and stir with a magnetic stir bar.
2. Trim the end a 1000 $\mu$ L pipet tip (remove about 2mm) and use it to SLOWLY draw the Tween (Tween is viscous and care must be taken to measure the correct amount).
3. Slowly dispense the Tween into the buffer and then pipet several times to rinse the Tween from the tip.
4. Add the required amount of NaCl and continue stirring to dissolve.
5. Transfer the buffer to a graduated cylinder and add 1X PBS to achieve the 1L final volume.
6. Transfer back to the transparent container and stir for at least 5 minutes.
7. Use a standardized pH meter to check the pH. The pH should be between 7.3 and 7.5.
8. If the pH is not within the acceptable range, consult with your QA Specialist.
9. If the pH is acceptable, pass the buffer through a 0.22 $\mu$ m membrane filter into a 1L bottle.
10. Label the bottle with the buffer name, preparation date, expiration date, and initials.
11. Store at 4°C for up to 2 weeks.

# MARIA<sup>®</sup> for Foods Extraction Protocol

## Method

1. Warm food extraction buffer to 60°C in a water bath or incubator for 30min prior to use.
2. Weigh out 1g of food and grind/chop/crush/mortar and pestle into smaller uniform pieces (if solid material).
  - a. Clean tools with 70% Isopropanol between each sample.
3. Add sample to a 15mL centrifuge tube and then add 10mL of heated food extraction buffer for a 1:10 ratio of food to extraction buffer.
4. Incubate samples at 60C in a shaking incubator or shaking water bath for 30 minutes
5. Centrifuge samples for 20 minutes at 2500rpm.
6. Pipet supernatant into 2mL cryovials and analyze samples immediately or store for 1 day at 4°C or longer term at -20°C.