SHORT INSTRUCTION

SENSI*Spec* Extraction Additive 100g

(Cat. nr. HU0030100)

Extraction additive for polyphenol-containing samples for use in combination with Gold Standard Diagnostics Allergen ELISAs and LFDs as well as for wine samples in combination with the Gold Standard Diagnostics Ochratoxin A RAPID ELISA

1. SAMPLE PREPARATION

Sample Preparation for Polyphenol-containing Food Samples in Combination with Gold Standard Diagnostics Allergen ELISAs

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be **cleaned thoroughly** before and after each sample. Proteins adhere strongly to different surfaces in some cases. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for all kinds of samples:

1) To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.

2) 1 g of the homogenized mixture is suspended together with 2 g of extraction additive in 20 mL of **pre-diluted** extraction buffer *(**NOTE**: for Casein ELISA and Milk ELISA the quantities should be as follows: 0.5 g of sample plus 1 g of extraction additive should be suspended together in 10 mL of pre-diluted extraction buffer)*. Afterwards the suspension is incubated for 15 min in a pre-heated water bath at 40° (Crustaceans and Molluscs ELISA) or 60°C (all other allergen ELISAs except the below mentioned). To ensure good homogeneity, the samples should be shaken every two minutes.

3) The samples are centrifuged for 10 minutes at ≥ 2500 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.

4) 100 µL of particle-free solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the **pre-diluted** extraction and sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

This process is **not** applicable for the Gold Standard Diagnostics Gliadin/Gluten ELISA, the Gold Standard Diagnostics INgezim Gluten R5 ELISA and the Gold Standard Diagnostics Total Soy Protein ELISA due to deviating extraction procedures.

Sample Preparation for Polyphenol-containing Food Samples in Combination with Gold Standard Diagnostics Gliadin/Gluten ELISA

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be **cleaned thoroughly** before and after each sample. Proteins adhere strongly to different surfaces in some cases. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for all kinds of samples:

1) To maximize homogeneity and representativeness of the sample collection, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2) 1 g of the homogenized mixture is suspended together with 2 g of extraction additive in 10 mL of 40% ethanol. Afterwards the suspension is mixed for further 5 min to ensure good homogeneity.
3) The samples are centrifuged for 10 minutes at 2500 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4) Afterwards the particle-free solution is diluted 1:50 in **pre-diluted** sample dilution buffer (for example 20 µL of solution in 980 µL sample dilution buffer).

Sample Preparation for Polyphenol-containing Food Samples in Combination with Gold Standard Diagnostics Total Soy Protein ELISA

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be **cleaned thoroughly** before and after each sample. Proteins adhere strongly to different surfaces in some cases. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for all kinds of samples:

1) To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2) 1 g of the homogenized mixture is suspended together with 2 g of extraction additive in 20 mL of **pre-diluted** extraction buffer.
3) Afterwards the suspension is incubated for 15 min in a preheated water bath at 100°C. To ensure good homogeneity, the samples should be shaken every two minutes.
4) The samples are centrifuged for 10 minutes at 2500 g or higher. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
5) The samples are diluted 1:5 in **ready-to-use** sample dilution buffer B for milk products and sausage or in **ready-to-use** sample dilution buffer A for all other samples (e.g. 100 µL prepared sample + 400 µL diluent).

Sample Preparation for Polyphenol-containing Food Samples in Combination with Gold Standard Diagnostics Allergen LFDs

Due to high risk of cross-contamination all applied instruments like applicator, mortar, vials etc. have to be **cleaned thoroughly** before and after each sample. Allergen proteins adhere very strongly to different surfaces. In certain cases, they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions for pattern recognition.

1) Homogenize sample using appropriate methods depending on its specific nature (e.g. grind, crush, mix).
2) *Solid samples:* Transfer one spatula of sample to an extraction tube. Alternatively, in order to increase precision, weigh out 0.2 g of sample into an extraction tube.

   Liquid samples: Transfer a half spatula of sample liquid to the extraction tube. Alternatively, in order to increase precision, pipette 0.2 mL of sample into an extraction tube.
3) Add 0.2 g of extraction additive into the extraction tube.
4) Add 3 mL of ready-to-use extraction buffer to the sample by using the disposable 3 mL pipette.
5) Close extraction tube with cap and shake for 1 minute.
6) Let the solid remains sediment. Depending on nature of the samples this might take 1-2 minutes. Alternatively centrifuge at 2000 g or higher.
7) Remove cap and transfer 0.3 mL of sample supernatant into a reaction vial using a disposable 0.3 mL pipette.

This process is **not** applicable for the Gold Standard Diagnostics Gluten LFD due to a deviating extraction procedure.

Sample Preparation for Polyphenol-containing Food Samples in Combination with Gold Standard Diagnostics Gluten LFDs

Due to high risk of cross-contamination all applied instruments like applicator, mortar, vials etc. have to be **cleaned thoroughly** before and after each sample. Allergen proteins adhere very strongly to different surfaces. In certain cases, they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions for pattern recognition.

1. Homogenize sample using appropriate methods depending on its specific nature (e.g. grind, crush, mix).
2. *Solid samples:* Transfer one and a half spatula of sample to an extraction tube. Alternatively, in order to increase precision, weigh out 0.3 g of sample into an extraction tube.

   Liquid samples: Transfer one spatula of sample liquid to the extraction tube. Alternatively, in order to increase precision, pipette 0.3 mL of sample into an extraction tube.
3. Add 0.2 g of extraction additive into the extraction tube.
4. Add 3 mL of ethanol (50%) to the sample by using one of the disposable 3 mL pipettes.
5. Close extraction tube with cap and shake for 1 minute.

6. Let the solid remains sediment. Depending on nature of the samples this might take 1-2 minutes. Alternatively centrifuge at 2000 g or higher.
7. Remove cap and transfer 0.3 mL of sample supernatant into a dilution vial using a disposable 0.3 mL pipette.
8. Add 3 mL of dilution buffer to the sample by using the second of the disposable 3 mL pipettes.
9. Close dilution tube with cap and shake for 1 minute.
10. Remove cap and transfer 0.3 mL of the mixture into an incubation vial using the same 0.3 mL pipette as in step 7.

Sample Preparation for Wine Samples in Combination with the Gold Standard Diagnostics Ochratoxin A RAPID ELISA

The following sample preparation should be applied for wine samples:

1) Adjust the pH of an adequate volume of wine to pH 7.0
2) Add 0.5 g of extraction additive to 10 mL of wine (pH 7.0) and mix suspension for 3 min.
3) Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
4) Dilute 1 mL of filtrate / supernatant wit 4 mL of 40% methanol and test the sample in the ELISA.

Gold Standard Diagnostics Budapest Kft.
Fóti út 56 A Ép., Budapest, Hungary
Tel: + 36 20 457 1204| Email: support.bud@eu.goldstandarddiagnostics.com