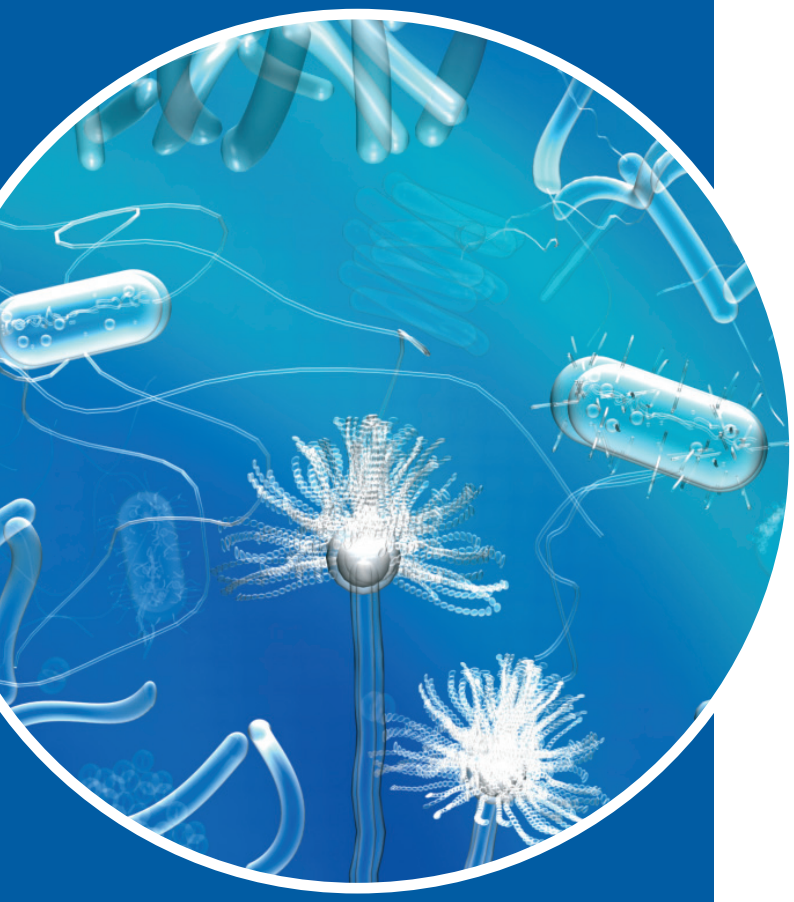
R-Biopharm AG

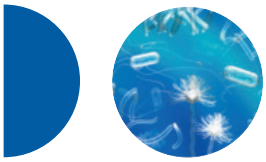


RIDASCREEN® SET Total

Art. No. R4105

- **Specific**
  Detects all Staphylococcus enterotoxins (A - E) which may be present in a food sample
- **Rapid**
  Results in less than 3 h
- **Sensitive**
  Limit of detection: 0.25 ng/ml toxin
- **Simple**
  Proven sandwich ELISA technology in microtiter plate format with 96 wells
  (12 x 8 removable wells)
- **Flexible**
  Can be evaluated visually or photometrically (automation possible)
- **Reliable**
  Tested and validated with all food categories which are known to be often contaminated with *S. aureus*

01/2010



RIDASCREEN® SET Total

Art. No. R4105

R-Biopharm contacts:

- **International Sales:**

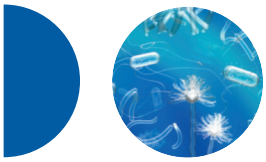
  Phone: +49 (0) 61 51 - 81 02-0
  E-mail: sales@r-biopharm.de

- **National Sales:**

  Phone: +49 (0) 61 51 - 81 02-0
  E-mail: info@r-biopharm.de

- **Orders:**

  E-mail: orders@r-biopharm.de



RIDASCREEN® SET Total

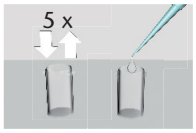
Art. No. R4105

Test procedure

1

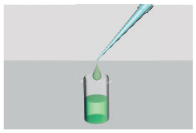
Binding of Staph. Enterotoxins present in the sample
- add 100 µl of controls or samples, respectively, to each well
- incubate at 35 - 37 °C for 1 h (cover plate)

2



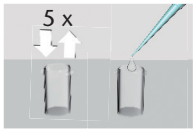
First washing
- wash each well 5 times with 300 µl washing buffer

3



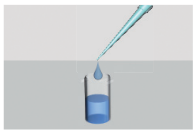
Adding Conjugate 1
- add 100 µl conjugate 1 to all wells
- incubate at 35 - 37 °C (95 - 99 °F) for 1 h (cover plate)

4



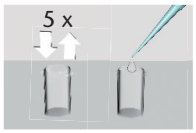
Second washing
- wash each well 5 times with 300 µl washing buffer

5



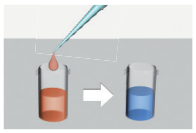
Adding Conjugate 2
- add 100 µl conjugate 2 to all wells
- incubate at 35 - 37 °C (95 - 99 °F) for 30 min

6



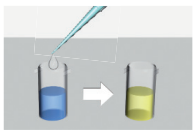
Third washing
- wash each well 5 times with 300 µl washing buffer

7



Adding Substrate/Chromogen and reading
- add 100 µl substrate/chromogen to each well
- incubate 15 min at 35 - 37 °C (95 - 99 °F) in the dark
- colour change from red to blue indicates positive samples

8



Adding Stop Solution and reading
- add 100 µl stop solution
- read absorbance at 450 nm with a microtiter photometer

Negative control (NC)	Positive control
< 0.100 OD units	≥ 1.000 OD units

Cut-off value	Negative sample	Positive sample
OD NC + 0.150 OD units	< cut-off	≥ cut-off

RIDASCREEN® SET Total

Art. No. R4105

Advantages of using RIDASCREEN® SET Total

- **Specific**

  Detects all relevant Staphylococcus enterotoxins (A-E) in food samples as well as bacterial cultures

- **Rapid:**

  Results in less than 3 h

- **Sensitive:**

  LOD: 0.25 ng/ml Toxin

- **Flexible:**

  Can be evaluated visually or photometrically. Using a fully automated EIA analyser is also possible

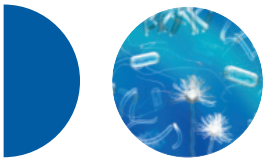
- **Approved:**

  Tested with relevant foods and pure bacterial cultures

- **Cost-effective:**

  Screening of samples with RIDASCREEN® SET Total saves money, time and material

Figure 1: Test kit RIDASCREEN® SET Total (R4105)



RIDASCREEN® SET Total

Art. No. R4105

Intended use

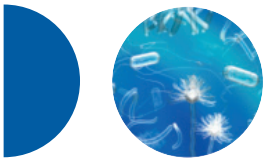
RIDASCREEN® SET Total is a sandwich enzyme immunoassay for the detection of total Staphylococcus enterotoxins (SET) A, B, C, D and E in fluid and solid foods as well as in bacterial cultures.

General

The main causative agent of food poisoning are enterotoxins of *Staphylococcus aureus* next to the intoxication with Salmonella. Among the strains of *Staphylococcus aureus* other Staphylococci species as *S. hyicus* and *S. intermedius* are able to produce one or more heat stable proteins which behave like enterotoxins.
Generally it is assumed that a population of 5 x 10^5 cells of enterotoxin-producing *Staphylococcus aureus* strains per gram of food is required to lead to an intoxication. However, other studies showed that only 100 - 200 ng of Staphylococcus enterotoxins can lead to symptoms of food poisoning.
SET intoxications have been frequently associated with pasta, finished meat products, ham, pies, chicken meat products, fish, fish products, milk, milk products, ice-cream, egg products, salads, pastries and cake stuffing as well as preparations from these food products. The enterotoxins of the serological groups A, B, C, D and E are very significant.

Test principle

RIDASCREEN® SET Total is a reliable test for detection of the most important toxins (A, B, C, D and E) of *S. aureus*. The surface of the microtiter plate is coated with specific, purified antibodies which can bind the enterotoxines contained in a sample.
Sample components not bound by the antibodies are then removed in a washing step. By adding specific marked antibodies against the toxins as well as enzyme marked detector molecules the sandwich complex will be formed (antibody-antigen-antibody-complex). After adding enzyme substrate/chromogen to the wells the bound enzyme conjugate converts the chromogen into a blue product. The results can be read visually or photometrically. A missing color reaction can be a sign for an enterotoxin free sample. After addition of the stop solution which leads to a colour change from blue to yellow the measurement can be made in a microtiter plate photometer.



RIDASCREEN® SET Total

Art. No. R4105

Basic Data

RIDASCREEN® SET Total is a sandwich enzyme immunoassay for the detection of total Staphylococcus enterotoxins (SET) A, B, C, D und E in one test well. Results will be obtained after a total testing time of 2 h 45 min (without sample preparation).

Specificity

The polyclonal antibodies included into the test system specifically detect enterotoxins A, B, C, D and E which will be build by *S. aureus* as well as some other species of Staphylococcus (e.g.: *S. hyicus* or *S. intermedius*).

Sensitivity

The limit of detection is at 0.25 ng/ml toxins.

Positive result

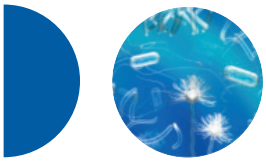
- **Visually evaluation:**

  The sample is considered to be positive if the negative control is clear or very pale blue and the sample is significantly deeper colored.

- **Photometrically evaluation:**
  Using a reader or automated EIA analyser the reaction has to be stopped by adding the stop solution prior to reading.
  The sample is considered to be positive if the measured absorption value is higher than the calculated cut-off value. The cut-off value is calculated by the optical density (OD) of negative control + 0.15 OD units.

Sample preparation

Sample preparation has to be performed as described within the product leaflet. Depending on the fat content either PBS-buffer or n-Heptane has to be used for homogenizing the samples. Homogenized samples have to be centrifuged refrigerated (10 °C / 50 °F). Prior to the use of 100 µl sample within RIDASCREEN® SET Total fat or n-Heptane supernatants have to be removed. Supernatants of bacterial cultures must be filtered with sterile filter equipment.



RIDASCREEN® SET Total

Art. No. R4105

Test validation

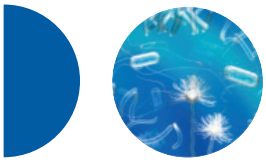
Limit of detection (LOD)

To determine the LOD 2 ng/ml of each of the five enterotoxins A, B, C, D and E had been solved in positive control buffer and diluted with the same buffer to the appropriate end concentrations. 100 µl of each dilution step have been used to perform RIDASCREEN® SET Total.

	SET A	SET B	SET C	SET D	SET E
Toxin concentration	OD_{450nm}	OD_{450nm}	OD_{450nm}	OD_{450nm}	OD_{450nm}
2 ng/ml	**2.379**	**2.361**	**2.261**	**2.445**	**3.249**
1 ng/ml	**1.361**	**1.314**	**1.265**	**1.343**	**1.798**
0.5 ng/ml	**0.755**	**0.672**	**0.678**	**0.711**	**0.925**
0.25 ng/ml	**0.363**	**0.346**	**0.335**	**0.343**	**0.445**
0.125 ng/ml	**0.179**	0.160	0.146	0.153	**0.199**
0.0625 ng/ml	0.073	0.073	0.067	0.064	0.090
Positivkontrolle	2.588	2.510	2.507	2.565	2.566
Negativkontrolle	0.019	0.018	0.017	0.017	0.016
cut-off-value (OD negative control + 0.15)	0.169	0.168	0.167	0.167	0.166

Table 1: Determination of LODs for single enterotoxins
ODs in bold types: positive values

Due to the measured values for concentration of 0.125 ng/ml for enterotoxins B, C and D the total LOD of RIDASCREEN® SET Total for detection of all enterotoxins has been set to 0.25 ng/ml.



RIDASCREEN® SET Total

Art. No. R4105

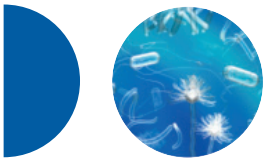
Test validation

Matrix effects / cross-reactions

Analysing food samples with immunological test system background effects deriving from the matrix often may be observed. To determine or exclude such effects several different unpolluted foods have been analysed with RIDASCREEN® SET Total.
Samples were prepared according to the description in the product leaflet. From the centrifugation supernatants 100 µl per sample were used for on single test.

	Sample 1	Sample 2	Average OD
Milk and dairy products	**OD_{450nm}**	**OD_{450nm}**	
UHT-Milk 3.5 % fat	0.020	0.016	0.018
Emmentaler 45 % fat of dry weight	0.018	0.019	0.019
French soft cheese 33.5 % fat of dry weight	0.019	0.021	0.020
Parmesan 28. 5 % fat of dry weight	0.024	0.021	0.023
Cream cheese (double cream) 25 % fat	0.020	0.020	0.020
Russian cheese 50 % fat of dry weight	0.017	0.017	0.017
Feta 21 % Fat	0.017	0.017	0.017
Russian cheese 45 % fat of dry weight	0.019	0.018	0.019
Gouda	0.019	0.019	0.019
Russian cows milk cheese 45 % fat of dry weight	0.017	0.017	0.017
Pepper soft cheese 70 % fat of dry weight	0.019	0.018	0.019
Russian cheese 45 % fat of dry weight	0.021	0.022	0.022
Russian semi-hard cheese 45 % fat of dry weight	0.023	0.022	0.023
Positive control	0.018	0.018	0.018
	2.640	2.651	2.646
cut-off value(average OD negative control + 0.15)	0.018	0.021	0.020

Table 2: Matrix effects of dairy products



RIDASCREEN® SET Total

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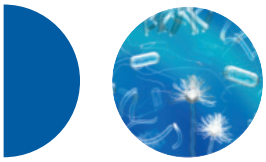
	Sample 1	Sample 2	Average OD
Fish and sausages	OD_{450nm}	OD_{450nm}	
Black Tiger Prawn	0.012	0.012	0.012
Plaice filet	0.034	0.046	0.040
Coalfish	0.014	0.013	0.014
Coalfish filet	0.116	0.101	0.109
Pikeperch	0.013	0.012	0.013
Catfish	0.028	0.041	0.035
Codfish	0.014	0.014	0.014
Trout	0.024	0.027	0.026
Sea devil	0.012	0.012	0.012
Salmon	0.015	0.018	0.017
Tuna	0.026	0.021	0.024
Shark catfish	0.045	0.049	0.047
Redfish	0.017	0.026	0.022
Saveloy 32 % Fett	0.018	0.022	0.020
ital. Salami 39 % fett	0.010	0.012	0.011
Turkey salami 17 % Fett	0.015	0.012	0.014
Westphalian Salami 32 % Fett	0.016	0.017	0.017
Canned sausage (Lab sample 1)	0.016	0.017	0.017
Canned sausage (Lab sample 2)	0.018	0.020	0.019
Positive control	2.056	1.939	1.998
Negative control	0.010	0.012	0.011
cut-off value (average OD negative control + 0.15)	---	---	0.161

Table 3: Matrix effects of fish and sausages

The presented data show that in the case of using RIDASCREEN® SET Total for analysis of relevant food samples no significant backgrounds effects can be observed. Cross-reactions with food proteins or other bacterial proteins are not known so far.



RIDASCREEN® SET Total

Art. No. R4105

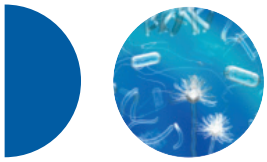
Test validation

Recovery

To test the recovery of enterotoxins in food matrices several different dairy products (s. Table 4) have been spiked with 1 ng/g SET E (2 ng/ml SET E respectively). Samples were prepared according to the description in the product leaflet. From the centrifugation supernatants 100 µl per sample were used for on single test. As positive control a concentration of 0.5 ng/ml toxin solved in positive control buffer has been used.

Milk and cheese samples – non-spiked	OD_{450nm}
Raw milk	0.018
UHT-Milk 1.8 % fat	0.021
UHT-Milk 3.8 % fat	0.022
UHT-skimmed milk 0.3 % fat	0.018
Russian semi-hard cheese 45 % fat of dry weight	0.039
Russian cows milk cheese 45 % fat of dry weight	0.035
Russian cheese 45 % fat of dry weight	0.050
Russian cheese 50 % fat of dry weight	0.035
Russian cheese 45 % fat of dry weight	0.071
Gouda 45 % fat of dry weight	0.016
French Soft Cheese 33.5 % fat of dry weight	0.020
Parmesan 28. 5 % fat of dry weight	0.018
Milk and cheese samples - spiked with 1 ng/ml SET E	**OD_{450nm}**
Raw milk	0.721
UHT-Milk 1.8 % fat	0.745
UHT-Milk 3.8 % fat	0.799
UHT-skimmed milk 0.3 % fat	1.111
Russian semi-hard cheese 45 % fat of dry weight	0.703
Russian cows milk cheese 45 % fat of dry weight	0.530
Russian cheese 45 % fat of dry weight	0.726
Russian cheese 50 % fat of dry weight	0.750
Russian cheese 45 % fat of dry weight	0.893
French Soft Cheese 33.5 % fat of dry weight	0.744
Cheese samples - spiked with 2 ng/ml SET E	**OD_{450nm}**
Gouda 45 % fat of dry weight	1.470
Parmesan 28.5 % fat of dry weight	1.179
Positive control (0.5 ng/ml)	0.472
Negative control	0.012
cut-off (OD Negative control + 0.15)	0.162

Table 4: Recovery of SET dairy products



RIDASCREEN® SET Total

Art. No. R4105

Test validation

Recovery

Concentrations of 1 or 2 ng/ml SET E, respectively, could be detected in all tested matrices. Measured optical densities were consistently higher than the value of the diluted (0.5 ng/ml) positive control.

Please note:

Quantification of total toxin by comparing with the absorption of the positive control is not possible.

Due to the fact that the solubility of toxin will differ depending on the food matrix, values of the spiked toxin will not be detected in relation to the positive control (e.g. double of OD-value at 1 ng/ml toxin concentration).

Stability of the test

Storage conditions

The kit should store at 2 - 8 °C (35 - 46 °F). The components should never be frozen. Unused micro wells have to be returned back to their original foil bag and resealed together with the included desiccant sachet. They can be further stored at 2 - 8 °C (35 - 46 °F) until required again.
The reddish colored substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light. All reagents, micro wells and enrichment broths have to be prewarmed to room temperature (20 - 25 °C/68 - 77 °F) prior to use.

Indication of deterioration of reagents

Any bluish colouration of the reddish substrate/chromogen solution prior to the test implementation indicates that the reagent is not useable anymore.

An absorption measured for the positive control which is below 1.0 (OD_{450nm} < 1.0) indicates that at least one of the test kit components is not useable anymore.

It is not possible to interchange individual reagents between kits of different production batches.