RIDASCREEN® SET Total

Art. No. R4105/R4106

Product Information

- **Specific**
  Detects the mainly occurring staphylococcal enterotoxins (A-E) in food samples
- **Simple**
  Proven Sandwich-ELISA technology in microtiter plate format
- **Approved**
  „Official European Screening Method for Staphylococcal Enterotoxins in Food"

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

Brief information

Technology:	Sandwich-ELISA
Format:	96 wells (R4105) or 48 wells (R4106) microtiter plate (12 x 8 removable wells)
Limit of detection (LOD):	0.25 ng/ml toxin per extract (equals to 0.375 ng/g)
Sample preparation:	"Official European Sample Preparation Method" (Dialysis Concentration Method) recommended

Background

The main causative agents 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 intoxication. However, other studies showed that only 100 - 200 ng of staphylococcal 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 enterotoxins 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 photometrical. A missing color reaction can be a sign for an enterotoxin free sample. After addition of the stop solution which leads to a color change from blue to yellow the measurement can be made in a microtiter plate

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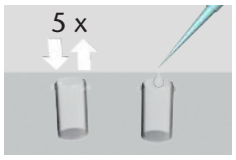
Test procedure

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)

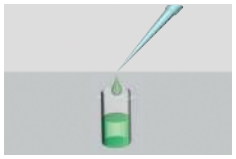
2



First washing

- Wash each well 5 times with 300 µl washing buffer

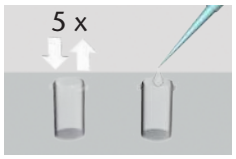
3



Adding Conjugate 1

- Add 100 µl conjugate 1 to all wells
- Incubate at 35 - 37 °C (95 - 99 °F) for 1 h (cover plate)

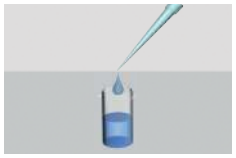
4



Second washing

- Wash each well 5 times with 300 µl washing buffer

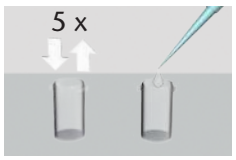
5



Adding Conjugate 2

- Add 100 µl conjugate 2 to all wells
- Incubate at 35 - 37 °C (95 - 99 °F) for 30 min

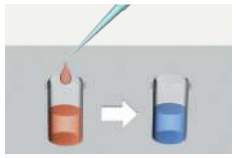
6



Third washing

- Wash each well 5 times with 300 µl washing buffer

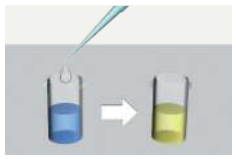
7



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

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Sample preparation

For sample preparation it is strongly recommended, to use the Dialysis Concentration Method (DCM) which was developed by the CRL-CPS, Maisons-Alfort, France. This preparation method is also described in the instructions for use (IFU) of RIDASCREEN® SET Total. Due to binding effects of SETs to the food matrices (s. page 7/8"Recovery Rates") the extraction of SETs from the sample can be difficult in a variety of cases. The detection (recovery) will be reduced as well. The DCM will eliminate interfering proteins and concentrate the extract so that very low toxin concentrations or uncomplete extraction can be compensated.
Nevertheless, there are simplified sample preparation methods (using an extraction with PBS buffer) described in the IFU, which can be used for matrices with high recovery rates in general or matrices which are known not to be contaminated by SETs or only in special cases. Depending on the fat content (40 % or higher) of the sample matrix, n-Heptane needs to be used for a first homogenization of samples to remove the fat.
Supernatants of pure cultures of potential toxin forming Staphylococcus strains need not to be treated by DCM, but must be filtered with sterile filter equipment prior to use in the RIDASCREEN® SET Total.

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.

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

	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
Positive control	2.588	2.510	2.507	2.565	2.566
Negative control	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

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.

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Matrix effects/cross-reactions

While analyzing food samples with immunological test systems, background effects deriving from sample matrices often may be observed. To determine or exclude such effects several different unpolluted foods have been analyzed with RIDASCREEN® SET Total.
Samples were prepared according to the simplified sample preparation method described in the IFU. From the final centrifugation supernatants 100 µl per sample were used for on single test.

Table 2: Matrix effects of dairy products

	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	2.640	2.651	2.646
Negative control	0.018	0.021	0.020
Cut-off value (average OD negative control + 0.15)	—	—	0.170

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Table 3: Matrix effects of fish and sausages

	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

The data presented in table 3 show that only fish as matrix tends to have an influence on the general background in the measurements, whereas other seafood or sausage products does not. In case of analyzing "coalfish filet", the background optical density (OD) was conspicuously different from the value observed within the negative control. In contrast the other "coalfish" sample showed no significant deviation from the OD value of the control. This leads to the suggestion that the observed backgrounds in several fish matrices might not only be due to the fish proteins in general but also to the individual freshness of the fish production batch!

On the scale of things the presented data in table 2 and 3 show that in the case of using RIDASCREEN® SET Total for analysis of food on presence of SETs, no significant background effects could be observed. Therefore the possibility of gaining false positive results within analyzing negative samples is quite low.
Only some slightly unspecific effects with the matrix fish were registered. Really measurable cross-reactions with food proteins or bacterial proteins are not known so far.

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Recovery rates

To test the recovery rates of enterotoxins in food matrices 57 different food products from 9 different categories (s. Table 4) have been spiked with combinations* of SEA/SEB/SED and SEC/SEE to end concentrations of 0.75 ng/g for each single toxin.
Samples were prepared according to the simplified preparation method described in the IFU, as the use of DCM would have prevented to generate “natural” recovery rates. From the supernatants of the final centrifugation step 100 µl per sample were used for on single test. As reference sample (100 % recovery) a concentration of 0.75 ng/ml toxin solved in positive control buffer was used.

Table 4: Recovery of SETs in 57 food matrices

Food		SET-recovery	
Category	Matrix	Toxin mixes, conc. 0.75 ng/ml each	
		A/B/D	C/E
Patisserie, chocolate	Milk chocolate	20.53 %	19.91 %
	Bittersweet chocolate	0.48 %	1.01 %
	"Mousse au chocolate" chocolate	18.03 %	18.19 %
Cakes	Chocolate-cream cake	55.10 %	52.46 %
	Raspberry-fresh-cheese cake	51.91 %	35.25 %
	Mini-cream-puffs	66.05 %	61.99 %
Eggs and noodles	Barn eggs	39.04 %	34.75 %
	Tortellini tuscany	68.22 %	62.66 %
	Spaghetti	88.01 %	87.58 %
	Tortellini	63.42 %	64.59 %
	Baguette tomato-mozzarella	67.56 %	64.91 %
Fish and seafood	Smoked salmon	15.57 %	16.42 %
	Smoked pepper mackerel	53.26 %	46.29 %
	Trout filet	53.12 %	46.43 %
	Coalfish filet	37.10 %	33.26 %
	Salmon filet	6.38 %	6.06 %
	Pacific plaice	7.03 %	6.84 %
	Ike-perch fillet	30.05 %	31.21 %
	Pacific deep-water prawn	35.51 %	33.29 %
	Rainbow trout	13.81 %	9.40 %
	Wild albacore	1.97 %	2.07 %
	Scallops (without shell)	40.78 %	42.28 %
Milk and dairy	UHT milk	132.40 %	126.10 %
	Emmental cheese	46.58 %	44.33 %
	French soft cheese	34.99 %	16.79 %
	Danish fresh cheese (double cream)	50.44 %	42.74 %
	Baby food with potatoes, carrots, beef	69.25 %	55.65 %
	Parmesan cheese	33.21 %	37.15 %
	Infant formula (powder)	24.80 %	16.34 %

* Combinations were designed according to known cross-reactions between SEA and SEA as well as SEB and SEC

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Table 4: Recovery of SETs in 57 food matrices (continued)

Food		SET-recovery	
Category	Matrix	Toxin mixes, conc. 0.75 ng/ml each	
		A/B/D	C/E
Vegetables	Canned mushrooms	70.43 %	56.73 %
	Canned maize	70.55 %	60.35 %
	Baby peas	66.38 %	57.15 %
	Tomatoes, minced in tomato juice	30.31 %	34.18 %
	Wryly cucumbers	11.54 %	10.31 %
	Red cabbage with apples	19.23 %	17.05 %
	Raw onion	43.12 %	34.85 %
Meat and sausages	Meat balls/patties	64.64 %	56.60 %
	Black pudding	62.77 %	66.59 %
	Tea sausage spread	34.61 %	41.93 %
	Ham	51.93 %	41.66 %
	Roast pork	51.89 %	49.75 %
	Smoked ham	21.43 %	22.56 %
	Cervelat sausage	24.60 %	20.05 %
	Liver sausage (pork)	57.54 %	49.64 %
	Meat from shoulder ham	54.58 %	49.78 %
	Turkey salami	7.72 %	3.35 %
	Calves liverwurst	56.27 %	51.98 %
Additives	Baking powder	50.58 %	57.21 %
	Blancmange powder	15.10 %	16.98 %
	Roma blancmange vanilla	50.64 %	50.41 %
Ready-to-eat food	Pea stew with sausage and bacon	62.01 %	56.96 %
	Goulash soup	54.91 %	48.80 %
	Vegetable stew	57.93 %	55.11 %
	Chili con carne	45.79 %	37.55 %
	Cabbage roll with potatoes	67.14 %	65.21 %
	German meatballs in caper sauce with potatoes	64.91 %	55.36 %
	Chicken breast with rice	69.25 %	56.86 %

Please note:

Quantification of total toxin in food samples by comparing with the absorptions of dilutions series of the positive control is not possible.
The matrix effects described above in table 4 prevent any quantification possibility of SETs, equal if naturally present in the food or artificially added.

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"Official European Screening Method"

The RIDASCREEN® SET Total has been tested by the Community Reference Laboratory for Coagulase-Positive Staphylococci (CRL-CPS) during an extensive ring trial with 30 participants from 12 different European countries. Since the beginning of 2013 the RIDASCREEN® SET Total is admitted as "Official European Screening Method for Staphylococcal Enterotoxins in Foods". The validation report is available on demand.

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 pre-warmed 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.

For further information or orders please contact R-Biopharm AG:

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