DRG® Pankrin ELISA (EIA-3781)

Revised 11 June 2007 **RUO in the USA**

INTENDED USE

With the DRG Pankrin ELISA an acute pancreatitis or an acute attack of a chronic pancreatitis is determined in subjects with abdominal pain. In the United States, this kit is intended for Research Use Only.

CLINICAL RELEVANCE

An acute pancreatitis, the acute inflammation of the pancreas, is mostly caused by the dystopic protease activation in the azinus cells, the obstruction of the luminal secretion and the formation of intracellular vacuoles (Schneider 1999). The yearly incidence rate is 50 to 100 cases per 100.000 persons. The clinical picture is determined by pronounced abdominal pain and general systems like nausea, vomiting, and circulatory collapse (Selberg *et al.* 1995). The mild form of an acute pancreatitis, the edematous pancreatitis (ca. 80% of all cases of an acute pancreatitis) progresses without complications and under adequate therapy patients normally recover within 72 hours. The severe form of an acute pancreatitis, the hemorrhagic necrotizing pancreatitis is characterized by necrosis and by a protracted course with frequent failure of organs or organ systems (Asanuma at. al. 1999, Singer *et al.* 1988). Morphologically a thickening and irregular delimitation of the pancreas can be observed as well as an accumulation of liquid in the pararenal space. The main cause of an acute pancreatitis is an increased alcohol consumption (Blank *et al.* 1999, Lankisch 2000) and trapped gallstones, which possibly get into the pancreatic duct via a reflux (Niederau *et al.* 1997, UHL *et al.* 1999, Sharma *et al.* 1999). Other pathogenetic factors are more rare. In a considerable percentage of the patients suffering from acute pancreatitis no risk factor can be detected. Cholelithiasis (gallstone disease) peaks between 40 and 60 years, the age maximum for alcohol abuse lies between 20 and 40 years.

FIELDS OF APPLICATION

In the functional diagnostics of the exocrine pancreas the DRG Pankrin ELISA is a sensitive and clinically easily practicable tool ideally complementing the sonographically established initial diagnosis.

PRINCIPLE OF THE TEST

The proteins detected by the DRG Pankrin ELISA are absolutely pancreas-specific. In marked contrast to clinical chemistry tests measuring only the enzymatic activity with the DRG Pankrin ELISA the pancreatic proteins immunologically, their immunological properties depend on the molecular structure. Therefore not only an absolute pancreas-specificity can be guaranteed but also it is also possible to measure already inactivated proteins in the serum. The pancreatic proteins getting into the serum during an acute attack can thus be detected for a much longer period of time than with conventional clinical chemistry test systems. The DRG Pankrin ELISA (Enzyme Linked ImmunoSorbent Assay) is a solid phase enzyme immunoassay based on the double sandwich technique. The wells are coated with polyclonal antibodies specifically recognizing pancreatic proteins. These antibodies immobilize Pancreatic proteins from the serum samples or standards. Then a secondary polyclonal antibody solution also specifically reacting with pancreatic proteins is added and binds to the pancreatic proteins immobilized on the first antibody layer in the well. In the following incubation step the biotin-residue of the bound biotin-antibody-complex reacts with an added peroxidase-streptavidin-complex which then enzymatically oxidizes the added substrate TMB (3,3',5,5'-tetramethyl-benzidine). This enzymatic activity is stopped after a defined time period by adding 0,25 mol/l H_2SO_4 and the amount of oxidized TMB is determined photometrically at 450 nm.

KIT COMPONENTS

(Sufficient for 96 determinations)

1. Microtiter strips coated with polyclonal anti-pancreas protein antibodies 96 wells
2. Standards
   standard 1, lyophilized (100 Pankrin-units/ml serum) - colourless cap

	standard 2, lyophilized (200 Pankrin-units/ml serum) - white cap	
	standard 3, lyophilized (400 Pankrin-units/ml serum) - yellow cap	
	standard 4, lyophilized (800 Pankrin-units/ml serum) - blue cap	
3.	Positive control, lyophilized (corresponding to 200 Pankrin-units/ml serum)	- green cap
4.	Biotinylated secondary polyclonal anti-pancreas protein antibodies - red cap	0,12 ml
5.	Streptavidin horseradish peroxidase conjugate (ready-to-use)	8 ml
6.	Washing buffer (10fold)	2x 50 ml
7.	Biotin conjugate dilution buffer	10 ml
8.	Substrate solution (TMB)	13 ml
9.	Stopping solution (0.25 mol/l H_2SO_4)	12 ml
10.	Holder for single strips	1 x

Store at 2 - 8°C. Please do not freeze!

GENERAL REMARKS

1. Avoid all contact with the stop solution.
2. Never pipette by mouth.
3. Warm all reagents to room temperature before use and mix thoroughly.
4. The optical density values of enzyme immunoassays depend on incubation time and incubation temperature. Therefore it is advisable to prepare all reagents before affecting the test (predilution of samples, fixation of the strips in the holder etc.). Only such a preparation guarantees equal time lapses for all incubation steps.
5. The reagents are stable until the expiry date indicated. Close tightly after use. Components of different kits or batches must not be mixed.
6. The microtiter strips are stored in resealable plastic bags with an exsiccating substance. Please reseal thoroughly after having taken out some strips. Sealed thoroughly the microtiter strips may be stored in the refrigerator for at least two weeks
7. The buffers and other solutions contained in the kits must not be mixed with additives (e. g., the addition of sodium azide for preservation would influence the enzyme reaction).
8. Once begun, the test has to be completed without interruption.
9. For optimal results it is important to wash the wells thoroughly after incubation and to remove even the last water drops by hitting the plate on absorbent paper or cloth.
10. Since the kinetics of the enzymatic reaction depend on the surrounding temperature different extinctions correlating with the respective room temperature may be observed. The optimal laboratory room temperature is 20 to 20 °C.
11. It is recommended to affect all tests in double determination in order to minimize the consequences of pipetting or handling errors.

REAGENTS AND DEVICES NOT INCLUDED IN THE KIT

- Microplate reader with a 450 nm filter, optionally a reference filter ≥ 550 nm
- Microtiter pipettes with disposable tips: 5 µl, 50 µl, 100 µl and 1000 µl
- Tubes for the dilution of the samples
- Aqua bidestillata
- Absorbent paper

SAMPLE MATERIAL
Serum

ATTENTION
There are no testing methods able to guarantee that no Hepatitis B-, Immunodeficiency Virus (HIV/HTLV-III/LAV) or other infectious agents are contained in the materials of the test kit. Please handle all human blood products and patient samples as potentially infectious. Please handle the samples and dispose of waste according to the respective national rules and laws. (e.g., USA: Center for Disease Control/ National Institute of Health Manual, Biosafety in Microbiological and Biomedical Laboratories", 1984).

TEST PROCEDURE
1. Warm all reagents to room temperature and mix thoroughly before use.
2. Preparation of the washing buffer:
   Dilute the washing buffer 1:10 (1+9) with aqua bidest. (for example: 50 ml buffer + 450 ml water). The diluted solution can be stored at 2 to 8 °C until the expiry date of the kit.
3. Preparation of the lyophilized standards:
   Reconstitute each lyophilized standard with 500 µl aqua bidest.. Do not use buffer! The reconstituted standards can be stored at 2 to 8 °C for up to four weeks.
4. Fix the required number of coated wells or strips in the strip holder. Fill in a paper form accordingly. Use up the microtiter strips within two weeks.
5. Dilute the serum samples 1:101 in washing buffer (for example: add 25 µl serum to 2.5 ml buffer). The reconstituted standards are ready-to-use.
6. Pipette 50 µl each of the zero standard (blank, washing buffer), the standards, the positive control und the diluted serum samples in the respective wells.
7. Incubate at room temperature for 60 min.
8. Discard the incubation solution. Wash the wells three times with 200 µl washing buffer each.
9. Dilute the biotinylated anti-pancreatic protein antibody solution 1:201 with biotin conjugate dilution buffer:
   5 µl anti-pancreatic protein antibody solution + 1 ml biotin-conjugate buffer
   10 µl anti-pancreatic protein antibody solution + 2 ml biotin-conjugate buffer
   15 µl anti-pancreatic protein antibody solution + 3 ml biotin-conjugate buffer
   25 µl anti-pancreatic protein antibody solution + 5 ml biotin-conjugate buffer
   and pipette 50 µl of this diluted solution into each well.
10. Incubate for 30 min at room temperature.
11. Discard the incubation solution. Wash each well three times with 200-µl-dilution buffer.
12. Pipette 50 µl of the ready-to-use streptavidin conjugate into each well.
13. Incubate for 30 min at room temperature.
14. Discard the incubation solution. Wash each well three times with 200 µl washing buffer each.

15. Pipette 100-µl substrate buffer into each well.
16. Incubate for 10 min at room temperature. Read the time when pipetting the substrate buffer into the first well.
17. Stop the colour development by adding 100-µl-stop solution per well.
18. Read the extinction in a microplate photometer at 450 nm. Optionally you may use a reference filter ≥ 550 nm, we recommend 630 nm, if available. The reference wavelength measurement is done to eliminate external contaminations of the plate, so you may well do without it.

EVALUATION OF THE TEST

1. Substract the OD (optical density) value of the blank (zero standard, only buffer) from the OD values of the standards, the positive control and the serum samples. Plot the optical density on the y-axis in relation to the concentration in Pankrin Units on the x-axis.
2. The resulting calibration curve is used to determine the values of the patient samples. The OD values of the serum samples are correlated with the corresponding Pankrin concentration values by interpolation.

RANGES OF NORMAL VALUES

Normal:	0 to 160 Pankrin-Units/ml serum
Uncertain:	161 to 190 Pankrin-Units/ml serum
Pathological:	> 190 Pankrin-Units/ml serum

These ranges were determined in a normal collective of 558 blood donors and 177 clinically clarified patients.

PIPETTING PATTERN

Blank	Blank	T	3	T	11	T	19	T	27	T	35
S	1	T	4	T	12	T	20	T	28	T	36
S	2	T	5	T	13	T	21	T	29	T	37
S	3	T	6	T	14	T	22	T	30	T	38
S	4	T	7	T	15	T	23	T	31	T	39
P	C	T	8	T	16	T	24	T	32	T	40
T	1	T	9	T	17	T	25	T	33	T	41
T	2	T	10	T	18	T	26	T	34	T	42

In this pipetting pattern the recommended positions for the standards (S1 – S4), the positive control (PC), and for the patient samples (T1 – T42) are shown as double determinations.