Kit for the radioimmunological detection of human trypsin (htrypsin).
For In Vitro Diagnostic use

Kit content:

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracer ≤ 90 kBq</td>
<td>1 x 22 mL</td>
</tr>
<tr>
<td>Anti-trypsin antiserum</td>
<td>1 x 10.5 mL</td>
</tr>
<tr>
<td>Calibrators</td>
<td>7 x qs 0.5 mL</td>
</tr>
<tr>
<td>Control serum</td>
<td>1 x 0.5 mL</td>
</tr>
<tr>
<td>Precipitation reagent</td>
<td>1 x 55 mL</td>
</tr>
<tr>
<td>Wash reagent</td>
<td>1 tube containing 1 tablet</td>
</tr>
<tr>
<td>Instruction For Use</td>
<td>1</td>
</tr>
</tbody>
</table>

**Warning:** Some reagents contain sodium azide
TRYPS-US

INSTRUCTIONS FOR USE

1. INTENDED USE

TRYPS-US is a radioimmunoassay for the quantitative determination of human trypsin (htrypsin) in serum. Trypsin quantification is a tool of interest in the differential diagnosis in presence of acute abdominal and other symptoms due to pancreatic disease.

2. INTRODUCTION

Differential diagnosis in patients presenting with "acute abdomen" is often difficult, particularly when the symptoms are due to pancreatic disease. Some means of assessing pancreatic function in these circumstances is desirable, and tests should also be suitable for monitoring the progress of the condition. In searching for a diagnostic marker, it is necessary to examine the characteristic products of the exocrine pancreas. In practice, these are the various pancreatic enzymes—principally, amylase, lipase, and trypsin which are involved in the intestinal digestion of carbohydrates, fats and proteins. Of these enzymes, only trypsin is produced solely by the pancreas and its measurement may thus be considered to provide an ideal basis for a specific test of pancreatic function.

Trypsin is one of the enzymes involved in the digestion of dietary protein in the small bowel. It functions as an endopeptidase with a marked preferential specificity for peptide bonds arising from the carbonyl groups of L-arginyl and L-lysyl residues. Chemically, trypsin is a protein consisting of 201 amino acids with a molecular weight of 22,900 Da.

In common with other proteolytic enzymes of pancreatic origin, trypsin is synthesized and secreted from the acinar cells of the exocrine pancreas as an inactive precursor or proenzyme, trypsinogen. This occurs in two forms, both of which are converted to enzymatically active trypsin by the action of enterokinase produced by cells in the duodenum. This kinase, in the presence of Ca++ ions, removes the hexapeptide Val-(Asp)4-Lys from trypsinogen, thereby unmasking the active centre of the trypsin molecule.

In addition trypsin itself can activate the trypsinogen by autocatalysis. Some trypsin appears to be excreted intact from the gut, as it can be detected and measured in stool. In addition, the trypsinogen, trypsin and probably trypsin bound to a pancreatic inhibitor may find their way from the pancreatic cells into the blood via the interstitial fluid. In blood all trypsin activity is blocked by three specific inhibitors (α1-antitrypsin, α2-macroglobulin and inter-α-trypsin inhibitor). Nevertheless, normal serum still retains some proteolytic activity as shown by the hydrolysis of synthetic substrates, but this cannot be due solely to trypsin.

3. PRINCIPLE OF THE ASSAY

Trypsin can be measured in duodenal or pancreatic juice by spectrophotometric methods, which depend upon its ability to hydrolyse synthetic substrates. However, as the trypsins are inactive and the enzymatic activity of trypsin in serum is blocked by the inhibitors mentioned above, this approach cannot be used to determine blood levels of the enzyme.

If the immunological rather than the enzymatic properties of trypsin could be used in a method for its determination in serum, the foregoing problem would not arise.

The Trypsin kit permits radioimmunological determination of the enzyme trypsin in human serum and other biological fluids. The kit utilizes the principle of competitive protein binding analysis, using a double antibody radioimmunoassay method, free trypsin being separated from antibody-bound trypsin by an anti-rabbit precipitating serum.

During the first incubation, serum trypsin and 125I-labelled trypsin compete for highly specific antibodies against trypsin, with the result that 125I-labelled trypsin is bound to the antibodies in inverse proportion to the amount of serum trypsin present.

During the second incubation the trypsin or 125I-trypsin antibody complex reacts with an anti-rabbit-gammaglobulin, causing precipitation. The precipitate can be centrifuged down, the supernatant decanted and the iodine-125 activity in the precipitate measured in a gamma scintillation counter. The activity measured is compared with a calibrator curve prepared under the same conditions and provides an indication of the trypsin concentration in the serum.

4. REAGENTS

The reagents supplied in one Trypsin kit are sufficient for preparation of a calibrator curve and measurement of 42 patients’ sera, in duplicate.

1 vial of 125I-trypsin (human), < 90 kBq, 22 mL buffer (water, Sodium dihydrogen phosphate dihydrate, Disodium hydrogen phosphate dihydrate, sodium chloride, chloridric acid, sodium hydroxide) with bovine albumin, rabbit IgG, sodium azide and an orange/red dye.

1 vial of rabbit anti-trypsin antiserum 10.5 mL buffer (water, Sodium dihydrogen phosphate dihydrate, Disodium hydrogen phosphate dihydrate, sodium chloride) with bovine albumin and sodium azide.

7 vials of htrypsin calibrators, per 0.5 mL freeze dried horse serum, human trypsin and sodium azide, concentration in the nominal range of 0-1,200 ng trypsin/mL.

1 vial of htrypsin control serum, 0.5 mL human serum and sodium azide, concentration stated.

1 vial of precipitation reagent, 55 mL of buffer (Sodium dihydrogen phosphate monohydrate, Disodium hydrogen phosphate dihydrate, Sodium chloride, EDTA, dextran, water), goat anti-rabbit IgG serum, bovine albumin and sodium azide.
1 tube of wash reagent (1 buffer tablet): Sodium dihydrogen phosphate monohydrate, Potassium Dihydrogen Phosphate, sodium chloride and potassium chloride

1 instruction for use.

* The values shown above are the target values. The real values are indicated on the label.

(*) The values shown above are only target values: the true value of each calibrator or control is shown on its label.

5. WARNING AND PRECAUTIONS

Safety measures
Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.
All animal products are derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
The dissolved reagents contain sodium azide as preservative. Avoid swallowing and contact with the skin or mucous membranes. Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

Basic radioprotection rules
This radioactive product may only be received, purchased, stored or used by persons so authorized and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user’s country.
The enforcement of the basic rules for handling radioactive products ensures adequate safety.
A summary of these is given below:
- Radioactive products must be stored in their original containers in a suitable area.
- A record of the reception and storage of radioactive products must be kept up-to-date.
- Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
- Do not eat, drink, smoke or apply cosmetics in a controlled zone.
- Do not use pipette radioactive solutions.
- Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
- Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
- Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
- All radioactive waste disposal must be carried out according to the regulations in force.

TRYPS-US kit is intended for in vitro diagnostic use.
TRYPS-US kit is intended for health professional only.
TRYPS-US kit is not intended to be used with automated systems

6. DILUTION AND RECONSTITUTION

Dilutions
No particular dilutions are required prior performing the assay

Reconstitutions
Calibrators: Carefully dissolve the calibrators in distilled water, proceeding as follows:
Gently tap the vials of calibrator to dislodge any particles which may be adhering to the stopper. Then carefully remove the stoppers, placing them upside down on the work surface. Using a suitable micropipette, dispense precisely 500 µL distilled water into each vial, replace the stoppers and allow approximately 10 minutes for the freeze-dried material to dissolve completely. Reconstitution and mixing may be accelerated by swirling the contents and/or rolling the vials between the hands.
Wash buffer: The wash buffer is prepared by dissolving the buffer tablet in 100 mL distilled water.

7. STORAGE INSTRUCTIONS

The kit is shipped at room temperature and should be stored at 2°-8°C. Keep away from heat or direct sunlight. The storage and stability period of each reagent (inclusive of reconstituted reagents) are indicated in section 4 of the instructions for use leaflet.

8. SPECIMEN COLLECTION AND PREPARATION

After blood sampling, serum is obtained by the usual methods. It is pipetted off, assayed directly or stored up to 3 days at +2/+8°C for later use, or alternatively frozen at -20°C.
The Trypsin radioimmunoassay kit permits measurement of trypsin as a protein in the serum, and is not affected by the presence of serum inhibitors. The radioimmunoassay is specific for trypsin, trypsinogen and the enzyme's inhibited forms.
9. **ASSAY PROCEDURE**

**Material required but not provided**
The following material is required but not provided in the kit (refer to section 4 for the list of reagents provided in the kit):
- Incubation tubes.
- Microlitre pipettes with disposable plastic tips (or Dispensettes) 100, 200, and 500 µL.
- Dispensers 1.0 mL.
- Measuring cylinders,
- Beaker.
- Rotary mixer,
- Centrifuge ≥ 1,5000 g,
- Gamma scintillation counter.

**Handling precautions**
- Before starting the assay, read completely and carefully the instructions for use. Use the version of the package insert provided with the kit. Be sure that everything is understood prior starting.
- Do not use kit components beyond their expiry date.
- Do not mix reagents from different batches.
- Follow good laboratory practices and safety guidances.
- Avoid any microbial contamination of the reagents or if the water used for washing.
- Follow reasonable precautions to avoid introduction of significant quantities of microorganisms.
- Fully respect the incubation times and the washing instructions.
- All the reagents should be stored at +2/ +8°C.

**Protocol**

1. Number sufficient incubation tubes (3-5 mL), as given in the Table (calibrators, control serum, serum samples and "total activity" tubes).

2. Pipette 100 µL calibrator, control serum and serum samples into test tubes that have been prepared.

3. Dispense 200 µL $^{125}$I-htrypsin into each test tube (including "total activity" tubes).

4. Dispense 100 µL rabbit anti-htrypsin serum into each test tube (excluding "total activity" tubes), mix the contents of the tubes on the rotary mixer, and incubate for 3h (3-5h) at room temperature (18-25 °C) away from direct light.

5. Dispense 500 µL precipitation reagent into each test tube (excluding "total activity" tubes), mix the contents of the tubes on the rotary mixer, and incubate for 30 min (30-60 min) at room temperature away from direct light.

6. Dispense 1mL of wash reagent into each test tube (excluding "total activity" tubes), centrifuge the tubes at ≥ 1,500 g for 15 min at 20 °C and decant the supernatants.

7. Measure the test tubes for 1 minute in a gamma scintillation counter. 40,000 to 20,000 cpm are to be expected as total activity.
Assay flowchart

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Labelling of the test tubes</td>
</tr>
<tr>
<td>2</td>
<td>Calibrators</td>
</tr>
<tr>
<td></td>
<td>CAL₁</td>
</tr>
<tr>
<td>Control serum</td>
<td>100/10</td>
</tr>
<tr>
<td>Sera</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>²²⁴-I-trypsin</td>
</tr>
<tr>
<td>4</td>
<td>Anti-trypsin serum</td>
</tr>
<tr>
<td></td>
<td>Mx ; incubate 3 h at 18-25 °C</td>
</tr>
<tr>
<td>5</td>
<td>Precipitation reagent</td>
</tr>
<tr>
<td></td>
<td>Mx ; incubate for 30 minutes at 18-25 °C</td>
</tr>
<tr>
<td>6</td>
<td>Wash reagent</td>
</tr>
<tr>
<td></td>
<td>Centrifuge for 15 min at ≥ 1,500 g, 20 °C, decant the supernatant</td>
</tr>
<tr>
<td>7</td>
<td>Measure the precipitate</td>
</tr>
</tbody>
</table>

Calibration
There is no internationally accepted calibrator for trypsin at present. The Trypsin values cannot be readily compared with the results of other trypsin assays. The calibrators and control values are assigned against normalized reference controls and the value of previous batch of calibrators and control.

The trypsin control serum included in the kit provides an analytical means of checking the accuracy of the assay procedure performed in the laboratory.

The calibrators range goes from 0 ng/mL to 1200 ng/mL.

In order to establish the calibration curve, the following procedure must be followed:
- Determine the mean value for counts of each duplicate for the calibrators 0 to 6 after subtracting the background.
- Express in counts per minute the bound activity of each calibrator, control and sample.
- Calculate the percent binding B/B₀(%) or in B/T(%) for each calibrator, sample and control as follows:

\[
B/B₀(%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100
\]

or

\[
B/T(%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Total Counts (T tubes)}} \times 100
\]

- The obtained B/B₀ (%) or B/T(%) of the calibrators (y-axis, linear) are plotted against their concentration (x-axis).
- For the calculation of the calibration curve, apply each signal of the calibrators (one obvious outlier of duplicates might be omitted and the more plausible single value might be used), the mathematical fit "hyperbole" must be applied in order to establish the calibration curve.

On occasions where large numbers of samples are to be assayed, reagents have to be pooled from more than one kit bearing the sample lot number. Only one calibration curve should be employed to interpolate all samples. It is possible to prepare 2 calibration curves with the reagents provided, but a consequently smaller number of serum samples can be measured.
The Figure shows a typical calibrator curve obtained with the Trypsin assay.

<table>
<thead>
<tr>
<th>Tube groups</th>
<th>Mean cpm</th>
<th>B/T x 100</th>
<th>B/Bo x 100</th>
<th>Concentration ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>35000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator 0</td>
<td>22225</td>
<td>63.5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Calibrator 1</td>
<td>19713</td>
<td>56.3</td>
<td>38.7</td>
<td>43</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>17002</td>
<td>48.5</td>
<td>76.5</td>
<td>85</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>13535</td>
<td>38.6</td>
<td>60.9</td>
<td>171</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>9779</td>
<td>27.0</td>
<td>44.0</td>
<td>341</td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>7445</td>
<td>21.2</td>
<td>33.5</td>
<td>602</td>
</tr>
<tr>
<td>Calibrator 6</td>
<td>5929</td>
<td>16.9</td>
<td>26.6</td>
<td>1365</td>
</tr>
<tr>
<td>Control</td>
<td>12050</td>
<td>34.5</td>
<td>54.4</td>
<td>220</td>
</tr>
</tbody>
</table>

Results:
Calculate the mean value from the two measured values of the patients’ sera, calculate their associated B/Bo(%) or B/T(%) values and the trypsin concentration per millilitre of serum is read off from the calibrator curve on the x-axis.

10. LIMITATION OF THE METHOD

It appears that the exocrine portion of the pancreas does not function as a unit after stimulation, as amylase, lipase and serum trypsin do not always correlate as expected. There may therefore be further possibilities for differential diagnosis. Normal serum trypsin concentrations (range 140-400 ng/mL) do not exclude the possibility of pancreatic impairment. High serum trypsins levels have also been found in renal insufficiency and the renal status of patients should therefore also be checked when interpreting trypsin levels. Bearing in mind the above limitation, trypsin levels below 140 ng/mL and above 400 ng/mL can be regarded as the lower and upper cut-off points of normality respectively when screening patients with abdominal symptoms.

11. EXPECTED VALUES

Normal levels
The function of enzymatic inactivated serum trypsin is not known, but trypsin can be detected in the serum as long as the exocrine portion of the pancreas is active. In healthy subjects the serum trypsin concentration ranges from 140-400 ng/mL (median 200 ng/mL). Trypsin levels do not vary significantly between normal males and females. But as serum trypsin levels in children appear to be lower than in adults, there does appear to be a degree of age dependency.

Chronic pancreatitis
The current methods available for the diagnosis of chronic pancreatitis include X-ray visualisation of calcification of the pancreas, determination of changes in amylase, lipase or bicarbonate levels in the duodenal fluid, or changes in the volume of fluid secreted. Some of these diagnostic procedures are very difficult to perform, are unreliable and unpleasant for the patient. The radioimmunoassay is therefore of great importance. It has been shown that the serum trypsin levels of patients with chronic pancreatitis are lower than 140 ng trypsin/ml in 50 % of cases, which is probably a result of extensive destruction of the acinar cells of the pancreas.
On the other hand, in 20 % of patients with chronic pancreatitis, the serum trypsin level exceeds the normal upper limit of 400 ng/mL. This probably indicates an acute phase of the disease. The severity of abdominal pain does not appear to correlate with tryps in serum levels, but steatorrhoea is frequently accompanied by a marked fall in serum trypsin.
In patients with chronic pancreatitis but "normal" trypsin basal levels, the serum trypsin increases markedly 20-120 minutes after stimulation with secretin followed by pancreozymin. This appears to be the result of increased permeability of the inflamed pancreatic tissue, since no such rise in serum trypsin is found in healthy patients undergoing the same test.

Acute pancreatitis
Patients with acute pancreatitis invariably have significantly increased serum trypsin concentration. The levels range between 600 and 6,500 ng trypsin/mL serum.

Carcinoma of pancreas
Patients with carcinoma of the pancreas may have moderately elevated serum trypsin levels (450-1,200 ng/mL) but generally there is no difference. If pancreatitis and/or renal insufficiency are excluded, other diagnostic procedures must be undertaken to confirm the diagnosis of carcinoma.
12. SPECIFIC PERFORMANCES

Precision
This was evaluated with 2 samples assayed 10 times in the same series and in 15 different series.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (ng/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>550</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>167</td>
<td>3.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (ng/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>162</td>
<td>4.9</td>
</tr>
<tr>
<td>4</td>
<td>483</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Interferences
The presence of bilirubin at concentrations of up to 250 mg/L, hemoglobin up to 10 g/L and triglycerides up to 20 g/L have no effect on the assay results. The immuno-assay is protected against heterophilic antibodies. However, we cannot guarantee that this protection is exhaustive.

Limit of detection
The detection limit is defined as being the smallest concentration different from 0 with a confidence interval of 95%. It has been determined as being 8.0 ng/mL.*

13. BIBLIOGRAPHY


14. SYMBOLS

<table>
<thead>
<tr>
<th>Meaning of symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE mark - Compliance with European Regulation</td>
</tr>
<tr>
<td>Storage temperature limitation</td>
</tr>
<tr>
<td>Batch number</td>
</tr>
<tr>
<td>Use by date</td>
</tr>
<tr>
<td>Read the instructions for use</td>
</tr>
<tr>
<td>In Vitro Diagnostic device</td>
</tr>
<tr>
<td>Manufactured by</td>
</tr>
<tr>
<td>Catalogue number</td>
</tr>
<tr>
<td>Number of determinations (the kit contains sufficient quantities of reagents for 100 determination)</td>
</tr>
<tr>
<td>Radioactive tracer</td>
</tr>
<tr>
<td>Calibrator</td>
</tr>
<tr>
<td>Incubation buffer</td>
</tr>
<tr>
<td>Antiserum</td>
</tr>
<tr>
<td>Immunoprecipitation reagent</td>
</tr>
<tr>
<td>Wash reagent</td>
</tr>
</tbody>
</table>
15. MANUFACTURER AND DISTRIBUTOR IDENTIFICATION & LAST REVISION

Cisbio Bioassays, located Parc Marcel Boiteux, BP84175 – 30200 Codolet – FRANCE, is the legal manufacturer of TRYPS-US kit. TRYPS-US kit is distributed in the US by ALPCO Diagnostic, Inc located 26-G Keewaydin Dr. Salem, NH 03079, USA.

Last revision of the instructions for use: version 13 from July 2016.