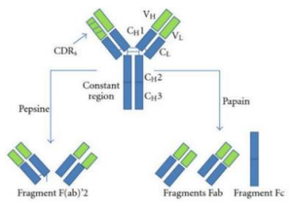
INTENDED USE

The **Carica papaya Papain** ELISA Kit is a sandwich ELISA for the detection and/or quantification of papain in solution. The kit is designed to measure trace residue of papain in vaccine, therapeutic antibodies, drugs or other biological samples, regardless of its enzyme activity; for research use only (RUO), not for diagnosis, cure or prevention of the disease.

GENERAL INFORMATION

Papain, also known as papaya proteinase I, is a cysteine protease (EC 3.4.22.2) enzyme present in papaya (Carica papaya) and mountain papaya (Vasconcelleacundina marcensis). The papain family of enzymes shows a wide variety of activities, including endopeptidases, aminopeptidases, dipeptidyl peptidases and enzymes with both exo- and endo-peptidase activity. Papain related enzymes are widespread, found in baculovirus (insect), eubacteria, yeast, and practically all protozoa, plants and mammals. The proteins are typically lysosomal or secreted, and proteolytic cleavage of the propeptide is required for enzyme activation. Papain-like cysteine proteinases are essentially synthesized as inactive proenzymes (zymogens) with N-terminal propeptide regions. The activation process of these enzymes includes the removal of propeptide regions, which serve a variety of functions in vivo and in vitro. Amino acid residues within the pro-region mediate their membrane association, and play a role in the transport of the proenzyme to lysosomes.

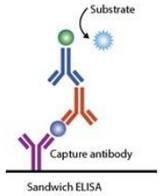


The papain precursor protein contains 345 amino acid residues, and consists of a signal sequence (1-18), a propeptide (19-133) and the mature peptide (134-345). The amino acid numbers are based on the mature peptide. The protein is stabilized by three disulfide bridges. Papain is a relatively heat-resistant enzyme, with an optimal temperature range of 60 and 70 °C. Papain breaks down tough meat fibers, and has been used for thousands of years to tenderize meat. Meat tenderizers in powder form with papain as an active component are widely sold. Papain can be used to dissociate cells in the first step of cell culture preparations. Papain is added to some toothpastes and mint sweets as a tooth whitener. Topical drug ointments (Accuzyme, Allanfil, Allanzyme, Ethezyme, Gladase, Kovia, Panafil, Pap Urea, and Ziox) containing papain are used to remove dead or contaminated tissue in acute and chronic lesions, such as diabetic ulcers, pressure ulcers, varicose ulcers, and traumatic infected wounds. Other products are marketed under the names of the active ingredients, for instance, papain-urea ointment. In 2008 the FDA announced its intention to take action against these products because it had received reports of serious adverse events in patients using products containing papain.

People allergic to latex can also be allergic to papaya, the source of papain, implying that people with latex sensitivity may be at increased risk of suffering an adverse reaction to a topical papain drug product. Therefore, sensitive immunoassay for papain are required to detect the presence of the enzyme even after inactivation.

An antibody digested by papain yields three fragments: two 50 kDa Fab fragments and one 50kDa Fc fragment. The papain-digested antibody is unable to promote agglutination, precipitation, opsonization, and lysis. Papain is used for fragmentation of many therapeutic antibodies (CroFab, DigiFab, DigiBind, ReoPro, Lucentis and Cimzia). Papain is routinely used for despeciation of anti-venom produced in horse, goat, and sheep.

PRINCIPLE OF THE TEST



The Papain ELISA kit is based on the binding of papain in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) conjugate. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of papain present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of papain in samples is calculated from a standard curve of purified papain of designated concentration.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.

KIT CONTENTS

To Be Reconstituted or Diluted: Store as indicated.

Component	Preparation Instructions
Sample Diluent Concentrate (20x) Cat.#. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. # WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Papain-HRP Conjugate Concentrate (100x) Part No. 800-164, 0.15ml	Peroxidase conjugated anti-papain in buffer with protein, detergents and BND as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Anti-Papain Coated Strip Plate	800-161	8-well strips (12)	Coated with purified anti-papain antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Papain Standards			
3 ng/ml	800-163B	0.65 ml	Five (5) vials, each containing Papain of designated concentrations; diluted in buffer with protein, detergents, and anti-microbial.
6 ng/ml	800-163C	0.65 ml	
15 ng/ml	800-163D	0.65 ml	
30 ng/ml	800-163E	0.65 ml	
60 ng/ml	800-163F	0.65 ml	
Positive Control [Papain] range on label	800-162	0.65 ml	Papain of stated concentration range; diluted in buffer with protein, detergents, and anti-microbial.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Culture medium, bioprocessing preparations, serum, and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference (See Limits of the Assay, page 6). For all samples, clarify by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks or frozen for long-term storage.

Assay Validation

Validate the performance of the sample antigen and matrix in the assay system for recovery and parallelism (see Limits of the Assay, page 6), as follows:

Recovery – a measure of the interference of the sample matrix (diluent effect) in providing accurate quantitation of the sample Humira relative to the Standard curve.

Prepare and run a series of dilutions of the sample antigen (concentrations that will fall within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. For most buffer solutions, a minimum 5-fold sample dilution is usually sufficient. Serum and plasma require at least a 10-fold dilution to obtain consistent quantitation or complete antigen recovery.

Parallelism – dilutions of the sample should read equivalent values from the top and bottom of the Standard curve to provide good assay precision.

Prepare a dilution series of the sample antigen that gives complete recovery and falls within the full range of the Standard curve. Sample readings from the upper and lower regions of the curve should differ by less than 25%.

Plate Set-up

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Prior to sample addition, add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-papain HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings and data calculations if available. Read absorbance of the entire plate at 450nm within 30 minutes after Stop Solution.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

CALCULATION OF RESULTS

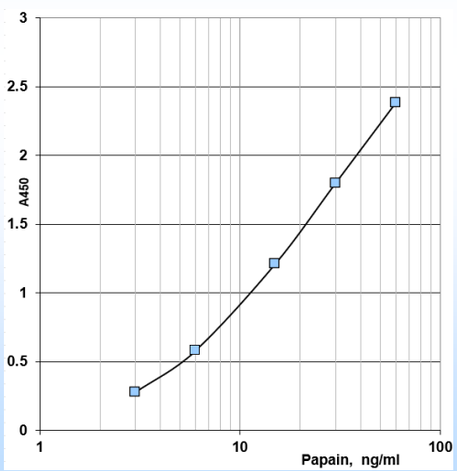
The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Papain concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Papain (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Papain concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor.
5. Samples producing signals higher than the 60 ng/ml standard should be further diluted and re-assayed.

Typical Results:

Wells	Standards & Controls		A450 nm
A1,2	**Negative Diluent Blank**		0.02
B1,2	3 ng/ml	**Standard**	0.28
C1,2	6 ng/ml	**Standard**	0.58
D1,2	15 ng/ml	**Standard**	1.21
E1,2	30 ng/ml	**Standard**	1.80
F1,2	60 ng/ml	**Standard**	2.38
G1,2	**Control**	[14 – 26 ng/ml]	1.42

Positive Control Result = **20.31** ng/ml



PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit have been affinity-purified using a papain immunosorbent and have been shown by immunoelectophoresis and ELISA to react specifically with papain.

Precision

Samples containing low, medium, and high concentrations of Papain as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

Papain concentrations were measured with good between-assay (4.3 to 9.2 %CV) reproducibility.

Sample	Papain ng/ml	Inter-assay % CV
High Concentration	35.4	**3.6**
Medium Concentration	19.2	**5.7**
Low Concentration	9.2	**6.1**

References: Cohen LW (1986) Gene 48, 219-227; Dretnh J (1968) Nature 218, 929-932; Rawligns ND (1994) Methd. Enzymol. 244, 461-486; Sebti SM (1987) Biochem. J. 290, 205-218; Yamamoto Y (2002) *Curr Protein Pept Sci* **3** (2): 231–238; Shuren J (2008) "Topical Drug Products Containing Papain; Enforcement Action Dates" (PDF). United States Food and Drug Administration, Department of Health and Human Services.

LIMITS OF THE ASSAY

1. Papain that is incomplete in sequence (truncated) or is aggregated and/or associated with other biomolecules may not produce dilution curves **parallel** with the Standard curve. For cases of non-parallelism, it may be useful to establish an alternative Standard curve using the altered papain preparation.

2. Papain in an activated buffer may require de-activation prior to its use as a sample in the assay, or proteolysis of the coating antibodies could produce falsely low signals. Generally, inactivation by removing sulfhydryl activators (e.g., by dialysis) is sufficient, but must be verified by the operator.

QUALITY CONTROL

Reagents

Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Standard Curve

The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-uniform or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. Do not rely on results generated from an assay with these issues.

Technique

Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing, and handling of samples and reagents.

Equipment

Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

Instruction Manual No. *M-800-160-CPP*

Papain ELISA Kit

Cat. 800-160-CPP, 96 Tests

For Quantitation of Papain in Solution

For research use only (RUO), not for diagnostic or therapeutic use.

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 W • San Antonio • Texas 78249 • USA

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com
www.4adi.com

ELISA Kit Components	Amount	Part
Papain Coated Microwell Strip Plate	8-well strips (12)	800-161
Papain Positive Control	0.65 ml	800-162
Papain Standard 3 ng/ml	0.65 ml	800-163B
Papain Standard 6 ng/ml	0.65 ml	800-163C
Papain Standard 15 ng/ml	0.65 ml	800-163D
Papain Standard 30 ng/ml	0.65 ml	800-163E
Papain Standard 60 ng/ml	0.65 ml	800-163F
Anti-Papain HRP Conjugate (100X)	0.15 ml	800-164
Sample Diluent (20X)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-800-160-CPP