CE

AccuDiag™ PR3 (c-ANCA) ELISA Kit

Cat#1445-1

IVD ⚠ See external Label 2°C—8°C Σ 96 Tests

Test	PR3 (c-ANCA) ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect; Antigen Coated Plate
Detection Range	Semi-Quantitative /Positive, Negative Cut off
Sample	10 µL
Total Time	~ 75 min.
Shelf Life	12 Months from the manufacturing date
Specificity	95.6%
Sensitivity	98.2%

INTENDED USE

The Diagnostic Automation Proteinase-3 (PR-3) Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection and semi-quantitative determination of antibodies to PR-3 in human sera. The assay is to be used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid in the diagnosis of Wegener's granulomatosis. **For in *vitro* diagnostic use. High complexity test.**

SUMMARY AND EXPLANATION

 Proteinase-3 is a lysosomal enzyme that can be found in human neutrophils (1,2). There are two subsets of autoantibodies to human neutrophils: c- ANCA and p-ANCA (3). The first subtype, c-ANCA, shows cytoplasm staining by IFA and is diagnostic for Wegener's granulomatosis (4,5). The major antigen responsible for the c-ANCA staining is Proteinase-3 (4,5). The second subtype, p-ANCA, shows perinuclear staining by IFA (4). The major antigen responsible for the p-ANCA response has been shown to be myeloperoxidase (6). The ANCA staining patterns are obtained using ethanol-fixed human neutrophils. Anti- PR-3 autoantibodies have been associated with Wegener's granulomatosis (4,5,7) but less often with microscopic polyangiitis (6,7). Other antigen specificity's (CAP 57) can be responsible for the c-ANCA pattern. These antigens are generally not diagnostic for PR-3 associated vaculitus diseases (7).

TEST PRINCIPLE

The DAI PR-3 test is an Enzyme-Linked Immunosorbent Assay to detect IgG, IgA, and IgM antibodies to PR-3 antigens. Purified PR-3 antigens are attached to a solid phase microassay well. Diluted test sera are added to each well. If the antibodies are present that recognize the antigen, antigen-antibody complexes are formed. After incubation, the wells are washed to remove unbound antibody. An enzyme labeled anti-human IgG, A, M, is added to each well. If antibody is present the conjugate will bind to the antigen-antibody complexes. After incubation, the wells are washed to remove unbound conjugate. A substrate solution is added to each well. If enzyme is present the substrate will undergo a color change. After an incubation period, the reaction is stopped and the color intensity is measured photometrically, producing an indirect measurement of specific antibody in the patient specimen. (10, 11, 12, 13).

SPECIMEN COLLECTION AND PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents (8).
2. Optimal performance of the DAI ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, nonlipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture (9). Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2° and 8° C if testing will take place within five days. If specimens are to be kept for longer periods, store at -20° to - 70° C in a non-defrosting freezer. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycle and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
5. Do not use heat inactivate sera.
6. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990) (9).

MATERIALS AND COMPONENTS

Materials provided with the test kits
Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.
1. **Proteinase-3 antigen coated microassay plate**: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
2. **Serum Diluent Type III**: Ready to use. Contains buffer, BSA and Tween-20, and proclin (0.1%) as a preservative. (96T: one bottle, 30 mL)
3. **High Positive Control**: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The High Positive Control is utilized to control the upper dynamic range of the assay. (96T: one vial, 0.4 mL) *
4. **Calibrator:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 mL) *
5. **Negative Control:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL) *
6. **Low Positive Control**: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with the established range printed on vial label. The Low Positive Control is utilized to control the range near the cutoff of the assay. (96T: one vial, 0.4 mL) *
7. **Horseradish-peroxidase (HRP) Conjugate:** Ready to use. Goat anti- human IgG, IgA, and IgM containing proclin (0.1%) and gentamicin as preservatives. (96T: one bottle, 15 mL)
8. **Wash Buffer Type II (20X concentrate):** Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-80 and proclin (0.1%) as a preservative. (96T: one bottle, 50 mL)
9. **Chromogen/Substrate Solution Type II**: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 mL)

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DAI CODE #1   Page 1 of 6

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10. **Stop Solution**: Ready to use, contains a 1N H2SO4 solution. (96T: one bottle, 15 mL)

* Note: serum vials may contain excess volume.

Materials required but not provided

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes Less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH$_2$0), CAP (College of American Pathology) Type 1 or equivalent (15, 16).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 Minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH$_2$0).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the Reference filter to 600-650 nm. Read the Operator's Manual or contact the Instrument manufacturer to establish linearity performance specifications of the Reader.

Note: Use only clean, dry glassware.

Preparation for the Assay

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21°- 25° C). Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type II to 1 L with distilled and/or deionized H$_2$0. Mix well.

ASSAY PROCEDURE

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Calibrator determinations (one Negative Control, three Calibrators, and one High Positive Control and one Low Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #2
1B	NC	2B	Patient #3
1C	Cal	2C	Patient #4
1D	Cal	2D	Patient #5
1E	Cal	2E	Patient #6
1F	HPC	2F	Patient #7
1G	LPC	2G	Patient #8
1H	Patient #1	2H	Patient #9

RB = Reagent Blank - Well without serum addition run with all reagents. Utilized to blank reader.
NC = Negative Control
Cal = Calibrator
HPC = High Positive Control
LPC = Low Positive Control

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum.)
3. To individual wells, add 100 µL of the appropriate diluted Calibrator, Controls and patient sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21° to 25° C) for **30 minutes +/- 2 minute.**
5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

IMPORTANT NOTE: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. **Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21° to 25° C) for **30 minutes** +/- **2 minute.**
8. Repeat wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21° to 25° C) for **15 minutes +/- 2 minute.**
11. Stop reaction by addition of 100 µL of Stop Solution (1N H$_2$SO$_4$) following the same order of Chromate/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to1 hour after addition of the stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is $\geq$ 0.150. The run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

RESULTS

1. Mean Calibrator O.D. - Calculate the mean value for the Calibrator from three Calibrator determinations. If any of the three Calibrator Values differ by more than 15% from the mean, discard that value and calculate the average of the two remaining values.
2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by DAI for each lot of kits. The Correction Factor is printed on the Calibrator vial.
3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in step 1.
4. Index Value - Calculate an Index Value for each specimen by dividing the specimen O.D. value by the Cutoff Calibrator Value determined in Step 3.

Example: O.D.s obtained for Calibrator = 0.38, 0.42, 0.40
Mean O.D. for Calibrator = 0.40

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DAI CODE #1   Page 2 of 6

Correction Factor = 0.50
Cutoff Calibrator Value = 0.50 x 0.40 = 0.20
O.D. obtained for patient sera = 0.60
Index Value = 0.60/0.20 = 3.00

Analysis
1. The patients' Index Values are interpreted as follows:

Index Value	Results	Interpretation
≤ 0.90	Negative	No detectable PR-3 antibody by the ELISA test.
0.91-1.09	Equivocal	Samples should be retested. See number 2 below.
≥ 1.10	Positive	Indicates presence of detectable antibody to PR-3 by the ELISA test.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method or test a new sample.
3. Index Values of > 8.44 should be reported as greater than 8.44.

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:
1. Calibrators and Controls must be run with each test run.
2. Reagent Blank must be < 0.150 O.D. (Optical Density) at 450 nm (when read against Air Blank).
3. The mean O.D. value for the Calibrator should be ≥ 0.300 at 450 nm (when read against Reagent Blank).
4. The Index Values for the High Positive, Low Positive, and Negative Controls should be in their respective ranges printed on the vials. If the control values are not within their respective ranges, the test should be considered invalid and should be repeated.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS C24A for guidance on appropriate Quality Control practices (14).
7. If above criteria are not met on repeat, contact DAI Technical Service.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity
The DAI PR-3 IgG,A,M ELISA kit was evaluated relative to IFA for ANCA. Forty sera were from patients diagnosed with Wegener's granulomatosis. Forty sera were from patients diagnosed with microscopic polyangiitis. One hundred and fifty-five sera were from normals with various ages, gender, and geographical areas. Table 1 summarizes the data. Note: if random IFA positive sera are selected, due to other disease states causing ANCA patterns not associated with PR-3, the sensitivity and specificity relative to IFA will not be as high.

Table 1
Sensitivity and Specificity of the DAI PR-3 ELISA to IFA

DAI PR-3 ELISA Kit		Positive ≥ 1.10	Equivocal 0.91-1.09	Negative ≤ 0.90	Total
Alternate	Positive	53*	0	1**	54
ELISA	Negative	8**	0	173***	181
Kit	Total	61	0	174	235

Relative Sensitivity = 53/54 = 98.2%
   95% confidence Interval = 94.5-100%
Relative Specificity = 173/181 = 95.6%
   95% confidence Interval = 92.5-98.6%
Relative Agreement = 226/235 = 96.2%
   95% confidence Interval = 93.7-98.7%

The 95% Confidence Intervals were calculated using the normal method.

*Fifty sera were from patients diagnosed with Wegener's granulomatosis or microscopic polyangiitis with a c-ANCA pattern. Two sera were from patients diagnosed with microscopic polyangiitis with a p-ANCA pattern. One sera was from a patient diagnosed with microscopic polyangiitis with ANA thus making the c-ANCA pattern impossible to read. ** Eight sera were from patients diagnosed with Wegener's granulomatosis or microscopic polyangiitis that were negative for ANCA.

***One serum was from a patient diagnosed with microscopic polyangiitis with a c-ANCA pattern.

****One hundred fifty-five sera were from normals that were negative for ANCA. Eighteen sera were from patients diagnosed with microscopic polyangiitis with either a p-ANCA pattern or ANA or negative for ANCA. The same group of clinical sera were tested on a commercially marketed ELISA device to determine the relative sensitivity and specificity to an alternate ELISA. Table 2 summarizes the data.

Table 2
Sensitivity and Specificity of the DAI PR-3 ELISA Relative to Alternate ELISA

DAI PR-3 ELISA Kit		Positive ≥ 1.10	Equivocal 091-1.09	Negative ≤ 0.90	Total
Alternate	Positive	58	0	5**	63
ELISA	Equivocal	1	0	11	12
	Negative	2*	0	158	160
	Total	61	0	174	235

Relative Sensitivity = 58/63 = 92.1%
95% confidence interval = 85.3 - 98.9% Relative Specificity = 158/160 = 98.8%
95% confidence interval = 97.0 - 100% Relative Agreement = 216/223 = 98.9%
95% confidence interval = 94.5 - 99.2%

The 95% Confidence Intervals were calculated using the normal method.

* Both serum were from patients diagnosed with microscopic polyangiitis.

** All five sera were from normals

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DAI CODE #1   Page 3 of 6

The clinical sera and the potentially cross-reactive sera were grouped and the clinical sensitivity and specificity of the PR-3 ELISA assay was calculated. Table 3 summarizes the data.

Table 3
Clinical Sensitivity and Specificity of PR-3 ELISA

DAI PR-3 IGG,A,M ELISA				
Index	Positive ≥ 1.10	Equivocal 0.91-1.09	Negative ≤ 0.90	Total
Wegener's granulomatosis	39	0	1	40
Microscopic polyangiitis	22	0	18	40
Other autoimmune sera	0	0	21	21
Normals	0	0	155	155
Total	61	0	195	256

Wegener's granulomatosis
Clinical Sensitivity = 39/40 = 97.5%
        95% Confidence Interval = 92.6 - 100%
Microscopic polyangitis
Clinical Sensitivity = 22/40 = 55.0%
        95% Confidence Interval = 39.3 - 70.7%
Other autoimmune sera
Clinical Specificity = 21/21 = 100%
        95% Confidence Interval = 85.9 - 100%
Normals
Clinical Specificity = 155/155 = 100%
        95% Confidence Interval = 98.1-100%
The 95% Confidence Intervals were calculated using the normal method. The 95% Confidence Intervals for the clinical specificities were calculated assuming one false positive.

Precision

The precision of the DAI PR-3 IgG,A,M kit was determined by testing nine different sera ten times each on three different assays. The data are summarized in Table 4. With proper technique, the user should obtain C.V.'s of less than 15%.

Table 4
Precision Data

Serum #	Assay 1 (n=8)			Assay 2 (n=8)			Assay 3 (n=8)			Inter Assay (n=24)		
	X	S.D.	C.V.	X	S.D.	C.V.	X	S.D.	C.V.	X	S.D.	C.V.
1	8.70	.634	7.29%	8.92	.479	5.37%	9.37	.465	4.96%	9.00	.589	6.52%
2	2.99	.345	11.54%	3.03	.211	6.96%	2.92	.201	6.89%	2.98	.256	8.59%
3	2.68	.289	10.81%	2.76	.145	5.25%	2.70	.144	5.32%	2.71	.200	7.38%
4	2.52	.177	7.04%	2.52	.163	6.47%	2.53	.242	9.54%	2.52	.190	7.54%
5	10.33	.344	3.34%	11.95	.293	2.45%	10.29	.200	1.95%	10.86	.837	7.71%
6	0.17	.074	42.79%	0.15	.056	37.79%	0.010	.076	80.36%	0.14	.074	53.76%
7	0.08	.037	47.16%	0.06	.039	68.22%	0.04	.037	84.50%	0.06	.039	65.51%
8	1.28	.079	6.13%	1.31	.071	5.37%	1.21	.079	6.53%	1.27	.086	6.74%
9	1.08	.059	5.45%	1.18	.105	8.92%	1.06	.083	7.91%	1.10	.097	8.82%

X = Mean Thyroglobulin Value
S.D. = Standard Deviation
C.V. = Coefficient of Variation

Linearity

The DAI PR-3 Index Values were determined for serial twofold dilutions of five positive sera. The Index Values were compared to log2 of dilution by standard linear regression. The data in Table 5 indicate that the assay is semi-quantitative.

Table 5
Linearity

Serum #	Neat	1:2	1:4	1:8	1:16	1:32	1:64	r
1	7.50	6.04	4.17	2.61	1.26	0.63		0.991
2	2.96	2.09	1.47	0.97	0.37			0.995
3	8.44	6.98	5.22	3.66	2.36	1.37	0.60	0.992
4	2.24	1.88	1.23	0.63				0.993
5	3.24	1.94	0.98	0.41				0.984

Cross-Reactivity

Sera containing antibodies to potentially cross-reactive antigens were assayed on the PR-3 ELISA. The data in Table 6 indicate that antibodies to alternate autoimmune antigens do not cross-react with the DAI PR-3 ELISA kit.

Table 6
Cross-Reactive Data

Serum #	Antibody Apecificity	PR-3 Index Value	Interpretation
1	Ro	0.09	-
2	Ro	0.11	-
3	Ro	0.14	-
4	La	0.07	-
5	La	0.05	-
6	La	0.08	-
7	Sm	0.24	-
8	Sm	0.30	-
9	Sm	0.36	-
10	RNP	0.14	-
11	RNP	0.09	-
12	RNP	0.16	-
13	Jo-1	0.05	-
14	Jo-1	0.20	-
15	Jo-1	0.12	-
16	Scl-70	0.13	-
17	Scl-70	0.07	-
18	Scl-70	0.11	-
19	dsDNA	0.38	-
20	dsDNA	0.24	-
21	dsDNA	0.36	-

EXPECTED VALUES

1. Antibodies to PR-3 are strongly associated with the c-ANCA pattern (cytoplasmic anti-neutrophil cytoplasmic antibodies) (3,7).
2. Antibodies to PR-3 are found in most cases (> 90%) of Wegener's granulomatosis (4,5). The PR-3 ELISA was tested with 40 patients with Wegener's granulomatosis. Thirty nine patients (97.5%) were found to be positive.
3. Antibodies to PR-3 are found in many cases (approximately one half) of microscopic polyangiitis (7). The PR-3 ELISA assay was tested with 40 patients with microscopic polyangitis. Twenty two Patients (55%) were found to be positive.
4. Antibodies to PR-3 are rarely seen in normal populations (4). The PR- 3 ELISA was tested with 155 normals. One hundred fifty-five were found to be negative.

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DAI CODE #1   Page 4 of 6

PRECAUTIONS

1. For in vitro diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human- based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2 (7).
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I, and Serum Diluent Type II. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21° to 25° C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur.**
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Avoid splashing or generation and/or samples could cause erroneous results.
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. **Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents**.
13. Certain reagents in this kit contain sodium azide for use as a preservative. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing proclin, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, immediately flush area with copious amounts of water.
15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
17. **Caution:** Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. Do not use Chromogen/Substrate Solution if it has begun to turn blue.
19. The concentrations of anti-PR-3 in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

STORAGE

1. Store unopened kit between 2° and 8° C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8° C. Unused strips must be immediately resealed in a sealable bag with desiccant, and returned to storage at 2° and 8° C. If the bag is resealed with tape, the wells are stable for 30 days. If the bag is resealed with a heat sealer, the wells are stable until their labeled expiration date.
3. Store HRP Conjugate between 2° and 8° C.
4. Store the Calibrator, High Positive, Low Positive and Negative Controls between 2° and 8° C.
5. Store Serum Diluent Type III and 20X Wash Buffer Type II between 2° and 8° C.
6. Store the Chromogen/Substrate Solution Type II between 2° and 8° C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer Type II at room temperature (21° to 25° C) for up to 5 days, or up to 1 week between 2° and 8° C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination. Do not use if evidence of microbial contamination or precipitation is present.

REFERENCES

1. **Falk, R.J., and J.C. Jennet.** 1988. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerolonephritis. New Engl. J. Med. 318:1651-7.
2. **Kallenberg, C.G.M., et al.** 1991. Autoimmunity to lysosomal enzymes: new clues to vasculitis and glomerulonephritis. Immunol. Today.12:61-4.
3. **Falk, R.J., et al.** 1990. Clinical Course of Anti-Neutrophil Cytoplasmic Autoantibody - associated Glomerolonephritis and Systemic Vasculitis. Am. Intern. Med. 113:656-63.
4. **Nolle, B., et al.** 1989. Anticytoplasmic Autoantibodies: Their Immunodiagnostic Value in Wegener's Granulomatosis. Am. Intern. Med. 111:28-40.
5. **Niles, J.L, et al.** 1989. Wegener's Granulomatosis Autoantigen is a Novel Neutrophil Serine Proteinase. Blood. 74:1888-93.
6. **Gallicchio, M.C. and J.S. Savige.** 1991. Detection of Antimyeloperoxidase and anti-elastase antibodies in vasculitides and infections. Clin. Exp. Immunol. 84:232-7.
7. **Hagen, E.C., et al.** 1993. Antineutrophil Cytoplasmic Autoantibodies: A Review of the Antigens Involved, the Assays, and the Clinical and Possible Pathogenetic Consequences. Blood. 81:1996- 2002.
8. **CDC-NIH Manual.** 1993. In: Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition. U. S. Dept. of Health and Human Services, Public Health Service. pp 9-12.
9. **National Committee for Clinical Laboratory Standards.** 1990. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture Approved Standard. NCCLS Publication H18-A.
10. **Engvall, E., and P. Perlman.** 1971. Enzyme-Linked Immunosorbent Assay, (ELISA) Quantitative Assay of Immunoglobulin G. Immunochemistry. 8: 871-874.
11. **Engvall, E., and P. Perlman.** 1971. Enzyme-Linked Immunosorbent Assay, ELISA. Peeters. H.,ed. In: Protides of the Biological Fluids. Proceedings of the Nineteenth Colloquium, Brugge, Oxford. Pergamon Press. pp 553-556.
12. **Engvall, E., K. Jonsson, and P. Perlman.** 1971. Enzyme- Linked Immunosorbent Assay. II. Quantitative Assay of Protein Antigen, Immunoglobulin-G, By Means of Enzyme- Labelled Antigen and Antibody- Coated tubes. Biochem. Biophys. Acta. 251: 427-434.
13. **Van Weeman, B. K. and A.H.W.M. Schuurs**. 1971. Immunoassay Using Antigen-Enzyme Conjugates. FEBS Letter. 15: 232-235.

14. **NCCLS.** 1991. National Committee for Clinical Laboratory Standard. Internal Quality Control

15. Testing: Principles & Definition. NCCLS Publication C24- A. http://www.cap.org/html/ftpdirectory/checklistftp.html. 1998. Laboratory General - CAP (College of American Pathology) Checklist (April 1998). pp 28-32.

16. **NCCLS.** 1997. National Committee for Clinical Laboratory Standard. Preparation and Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3- A3.

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DAI CODE #1   Page 6 of 6