

**AccuDiag™
 Mitochondria
 ELISA Kit**

Cat#1420-1



Test	Mitochondria ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect; Antigen Coated Plate
Detection Range	Qualitative /Positive, Negative Cut off
Sample	10 µL
Total Time	~ 60 min.
Shelf Life	12 Months from the manufacturing date
Specificity	100%
Sensitivity	100%

INTENDED USE

The Diagnostic Automation Mitochondria Enzyme- Linked Immunosorbent Assay (ELISA) is intended for the detection and semi-quantitation of antibodies to mitochondria 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 to the diagnosis of primary biliary cirrhosis. **For in vitro diagnostic use. High complexity test.**

SUMMARY AND EXPLANATION

Systemic autoimmune disease is characterized by the presence of circulating auto-antibodies directed to a wide variety of cellular antigens (1,2,3). Systemic lupus erythematosus (SLE), commonly referred to as Lupus is the best known of these diseases. Other possible connective tissue diseases include mixed connective tissue disease (MCTD), Sjogren syndrome, scleroderma, and polymyositis/dermatomyositis. The majority can be diagnosed by clinical presentation and their antibody profiles to the various antigens involved, which include dsDNA, Sm, RNP, Ro, La, Scl-70, Jo-1 and Histones. Therefore, immunoassays for autoantibodies are useful for diagnostic and prognostic evaluations of autoimmune disease (1,2,3).

Mitochondria (M2) antigen is part of the pyruvate dehydrogenase complex (4). Approximately 90-95% of patients with primary biliary cirrhosis (PBC) have antibodies to M2. Antimitochondria antibodies occur occasionally in other liver conditions and scleroderma. The antibody is rarely seen in other conditions (5,6).

Classically, antibodies to autoimmune antigens are detected by double immunodiffusion. However, the test is lengthy and suffers from weak sensitivity. Enzyme-Linked Immunosorbent Assays (ELISAs) combine greater sensitivity with ease of use. Many ELISAs have been developed and validated for detecting autoantibodies to various antigens (7,8,9,10).

TEST PRINCIPLE

The DAI Mitochondria test is an Enzyme-Linked Immunosorbent Assay to detect IgG, IgA, and IgM antibodies to Mitochondria antigens. Purified Mitochondria antigens are attached to a solid phase microassay well. Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen- antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG,A,M conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H₂SO₄, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader. (13, 14, 15, 16)

SPECIMEN COLLECTION AND PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents (11).
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 (12). 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° C 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 cycles 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 - Procedure for the Handling and Processing of Blood Specimens, H18-A. 1990) (12).

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. **Mitochondria (M2) 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 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 dynamic 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 established range



printed on vial label. The Low Positive Control is utilized to control the negative 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 I:** 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)
10. **Stop Solution:** Ready to use, contains a 1N H₂SO₄ solution. (96T: one bottle, 15 mL).

* **Note: serum vials may contain excess volume.**

The following components are not kit lot # dependent and may be used interchangeably within the DAI ELISA Autoimmune Kits: Serum Diluent Type III, Chromogen/Substrate Solution Type I, Wash Buffer Type II, and Stop Solution. Please check that the appropriate DAI Reagent Type (Type I, Type II, etc.) is used for the assay.

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 (dH₂O), CAP (College of American Pathology) Type 1 or equivalent (18, 19).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 – 60 Minutes). Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH₂O).
10. 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 H₂O. 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.
4. Incubate each well at room temperature (21° to 25° C) for **30 minutes +/- 1 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 +/- 1 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 +/- 1 minute.**
11. Stop reaction by addition of 100 µL of Stop Solution (1N H₂SO₄) 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 to 1 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 ≥ 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
 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 antibody to Mitochondria 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 Mitochondria by the ELISA test.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method or test a new sample.

QUALITY CONTROL

1. For the assay to be considered valid the following conditions must be met: 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 (17).
7. If above criteria are not met on repeat, contact DAI Technical Service.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The DAI Mitochondria ELISA kit was evaluated relative to a commercially available ELISA test kit. Table 1 summarizes the data.

Table 1
Sensitivity and Specificity of the DAI Mitochondria ELISA Kit

DAI Mitochondria ELISA Kit					
Alternate ELISA Kit		Positive ≥ 1.10	Equivocal 0.91-1.09	Negative ≤ 0.90	Total
		Positive > 25	17	0	0
Equivocal 21-25	0	0	0	0	
Negative < 21	0	2	122	124	
Total	17	2	122	141	

Sera falling in the equivocal range were excluded from the following calculations.

Relative Sensitivity = 17/17 = 100%
 Relative Specificity = 122/122 = 100%
 Relative Agreement = 139/139 = 100%

Reproducibility

The precision of the DAI Mitochondria ELISA kit was determined by testing seven different sera eight times each on three different assays. The data are summarized in Table 2. With proper technique the user should obtain C.V.'s of less than 15%.

Table 2
Precision Data for the DAI Mitochondria ELISA Test

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	17.70	0.587	3.32%	17.00	1.03	6.07%	15.84	0.899	5.68%	16.85	1.14	6.77%
2	16.09	0.532	3.31%	15.69	0.804	5.12%	15.00	0.396	2.64%	15.59	0.735	4.71%
3	16.59	0.703	4.24%	16.67	0.590	3.36%	15.76	1.46	9.26%	16.34	1.04	6.36%
4	2.36	.0285	12.1%	2.28	0.269	11.8%	2.43	0.251	10.3%	2.36	0.264	11.2%
5	4.71	.0597	12.7%	4.41	0.518	11.8%	5.23	0.535	10.2%	4.78	0.630	13.2%
6	.290	0.092	31.7%	.251	0.169	67.3%	.264	0.115	43.6%	.068	0.124	46.3%
7	.330	0.141	42.7%	.373	0.179	48.0%	.404	0.205	50.7%	.369	0.172	46.6%

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

Linearity

The DAI Mitochondria 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 3 indicate that the assay is semi-quantitative.



Table 3
Linearity for the DAI Mitochondria ELISA Test

Serum #	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	r ²
1	5.71	5.18	4.79	3.93	3.36	2.21	1.29	.790	0.984
2	7.79	6.07	3.57	2.07	1.36	0.64			0.948
3	3.00	2.29	1.86	1.50	1.36	1.14	0.86		0.938
4	3.36	2.64	2.07	1.57	1.21	0.93			0.974
5	4.09	3.78	2.89	2.27	2.09	1.86	1.43	1.13	0.957

r² = coefficient of determination

Cross-reactivity

Sera containing high level of antibodies to potentially cross-reactive antigens were assayed on the DAI Mitochondria ELISA kit. The data in Table 4 indicate that antibodies to alternate autoimmune antigens do not cross-react with the DAI Mitochondria ELISA kit.

Table 4
Cross-reactivity Data for the DAI Mitochondria ELISA Test

Serum #	Antibody Specificity	Mitochondria Index Value	Interpretation
1	Sm	0.39	-
2	Sm	0.04	-
3	Sm	0.06	-
4	RNP	0.25	-
5	RNP	0.07	-
6	RNP	0.01	-
7	Ro	0.18	-
8	Ro	0.82	-
9	Ro	0.02	-
10	La	0.23	-
11	La	0.36	-
12	La	0.07	-
13	Scl-70	0.47	-
14	Scl-70	0.13	-
15	Scl-70	0.18	-
16	Jo-1	0.40	-
17	Jo-1	0.36	-
18	Jo-1	0.10	-
19	dsDNA	0.16	-
20	dsDNA	0.34	-
21	dsDNA	0.11	-

LIMITATION OF PROCEDURE

- The result of the assay should not be interpreted as being diagnostic. The results should only be used as an aid to diagnosis. The results should be interpreted in conjunction with the clinical evaluation of the patient
- Sera from patients with other autoimmune diseases and from normal individuals may contain autoantibodies.
- The assay should be used only with serum. Icteric, lipemic, hemolyzed and heat inactivated serum should be avoided.
- Index Values of ≥ 10.00 should be reported as greater than 10.

EXPECTED VALUES

- From 1-5% of apparently normal individuals may contain autoantibodies (1,2,3).
- Antibodies to M2 are found in approximately 90-95% of patients with primary biliary cirrhosis (PBC) (5,6).
- Antimitochondria antibodies occur occasionally in other liver conditions and scleroderma (5,6).

- Antibodies to mitochondria are rarely seen in other conditions (5,6).

PRECAUTIONS

- For in vitro diagnostic use.
- 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.
- 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).
- 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 III. Do not mix with components from other manufacturers.
- Do not use reagents beyond the stated expiration date marked on the package label.
- 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.**
- Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
- Use only distilled or deionized water and clean glassware.
- Do not let wells dry during assay; add reagents immediately after completing wash steps.
- Avoid cross-contamination of reagents. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause false results.**
- 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.
- 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.**
- 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. 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.
- 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.
- Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
- 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.
- Do not use Chromogen/Substrate Solution if it has begun to turn blue.
- The concentrations of anti-Mitochondria in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

The safety data sheet is available upon request.

WARNING



Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300R, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

P280: Wear protective gloves / protective clothing / eye protection / face protection.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed

P264: Wash thoroughly with plenty of soap and water after handling

P270: Do not eat, drink or smoke when using this product

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

P330: If swallowed, rinse mouth

P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

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 between 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 Control 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 Chromate/Substrate Solution Type I 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 one 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.

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2015-03-01	AccuDiag™- Mitochondria ELISA -2015		
<table border="1" style="display: inline-table;"> <tr> <td>EC</td> <td>REP</td> </tr> </table>	EC	REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
EC	REP		
Revision C Date: 2015-09-25			