Wantai SARS-CoV-2 RT-PCR Kit

Nucleic Acid Detection Kit for Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (PCR-Fluorescence Probing)

Instructions for Use

REF WS-1248 V. 2020-01 [Eng.] 48 IVD

【Intended use】 This kit is intended for qualitative detection of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA extracted from throat swabs, sputum, bronchoalveolar lavage fluid, aspirate in trachea samples of patients suspected for infection with COVID-19.

Infection with SARS-CoV-2 can cause the respiratory disease COVID-19. Its symptoms include fever, weakness and dry cough, and breathing problems that appear gradually. In severe cases acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis that is hard to correct, coagulation disorders and death.

【Test principle】 This kit is a qualitative, real-time fluorescent PCR in which specific primers and fluorescent probes are designed to detect the highly conservative regions of the ORFlab and N genes of the virus. This kit has integrated quality control (IC, human housekeeping gene) intended for monitoring of the test run.

【Components】

Amplification and controls	RT-PCR master mix	1.25mL×1	dNTPs, rTth enzyme, UDG enzyme
	$Mn(OAc)_2$	125μL×1	$Mn(OAc)_2$ solution
	Primer and probe	125μL×1	Primer and probe solution
	Positive control	1 mL×1	Artificial virus containing SARS-CoV-2 amplification target sequence
	Negative control	1 mL×1	Normal saline
Extraction	This kit does not contain RNA extraction reagents. Suggested extraction kits and equipment: Beijing Wantai, GenMagBio or QIAGEN and commercialized RNA extractor reagent kit with reliable quality.		

【Storage and shelf-life】

- Store the kit under -15°C. Avoid exposing the kit to direct sunlight. Do not press the package.
- Shelf-life 12 months.
- After opening the kit can be stored at -15°C for 6 week, freeze-thaw no more than 4 times.
- The kit can be transported at -15°C packed into a foam box with ice bags or dry ice.
- See the label for production and expiration date.

【RT-PCR Instruments】 Fluorescent qPCR: BIO-RAD CFX96, ABI 7500 and its upgraded version series.

【Sample requirements】 **Upper respiratory tract sample:** Oropharyngeal and nasopharyngeal swab. The swab should be a special purpose microbial swab (do not use common swabs). The head of the swab should be of medical grade artificial fiber, the material of the shaft should be plastic.

【Sample collection】 **Nasopharyngeal swab:** Use a microbial swab to collect samples in the nasal area. Softly rotate and push the swab, insert the head of the microbial swab deep into the nasopharynx at the root of the nasal cavity, rotate a few times to obtain an abundant sample. See image 1.

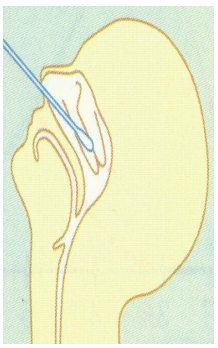


Image 1: nasopharyngeal sampling

Oropharyngeal swab: Use a microbial swab to wipe the posterior pharyngeal wall and tonsil on both sides with moderate force. Avoid touching the tongue.

Sample processing: After collecting the sample, insert the microbial swab into the sterilized tube containing the sampling liquid. Rotate several times against the inner wall of the tube to dissolve the sample in the solution as much as possible.

Lower respiratory tract sample: Sputum, endotracheal aspirates, bronchoalveolar lavage fluid. Add 4% NaOH in 2:1 proportion to the collected sputum or tracheal aspirate sample. Vortex to mix well then place at room temperature for 20 minutes to liquify. Liquification time can be

increased if there are too many viscous substances. Transfer 1 mL of liquified sample to a 1.5 mL centrifuge tube and then vortex again. If bronchoalveolar lavage fluid is clear, it can be directly used for nucleic acid extraction.

Sample storage and transportation: Samples tested within 12 hours after collection can be stored at 2-8°C. For long-term storage, keep under -70°C. Avoid multiple freeze-thaw cycles (no more than 3 times). Sample should be transported under -15°C. Before testing, balance the samples at room temperature. The frozen samples should be mixed well before testing.

【Testing Method】

【Reagents preparation】 PREPARATION AREA

- **STEP. 1 - PREPARE THE REAGENTS:** open the kit and remove the components from the box. Thaw at room temperature, shake to mix for 1 minute then centrifuge immediately. Place the RT-PCR master mix, $Mn(OAc)_2$ and primer probe at 2～8℃ refrigerator for later use.
- **STEP. 2 - PREPARE THE PCR REACTION MIX:** one test requires 30μL of PCR reaction mix. Depending on how many specimens will be tested, mix the required volumes of reagents as per the below table. Centrifuge intermediately after sufficient mixing. It is advised to prepare one additional test reagent each time to prevent loss of the reaction mix because of splitting.

Component	Volume per 1 reaction(μL)	Volume for 16 reactions(μL)	Volume for 32 reactions(μL)	Volume for 48 reactions(μL)	Volume for n reactions(μL)
RT-PCR master mix	25	400	800	1200	25×（n+1）
Primer probe	2.5	40	80	120	2.5×（n+1）
Mn $(OAc)_2$	2.5	40	80	120	2.5×（n+1）
Total	30	480	960	1440	30×（n+1）

- **STEP. 3 - TRANSFER TO PCR REACTION TUBE:** pipette 30μL of the PCR reaction mix into a PCR reaction tube (choose a PCR reaction tube compatible with the extractor instrument).
- **STEP. 4 - ADD THE RNA TEMPLATE:** add 10μL of RNA template or controls to the PCR amplification tube. Close the tube and centrifuge instantly. Transfer to the amplification and analysis area for PCR amplification. *(This kit does not contain RNA extraction reagents. Suggested extraction kits and equipment: Beijing Wantai, GenMagBio or QIAGEN and commercialized RNA extractor reagent kit with reliable quality.)*

【Amplification】 AMPLIFICATION AND ANALYSIS AREA

- Place the PCR amplification tube into the RT-PCR instrument.
- Label to indicate the controls and the samples positions.
- Select FAM for ORF1a gene, VIC /HEX for the N gene, and ROX for the IC.
- Set the PCR reaction mix volume to 40μL
- Set the cycles according to the table below:

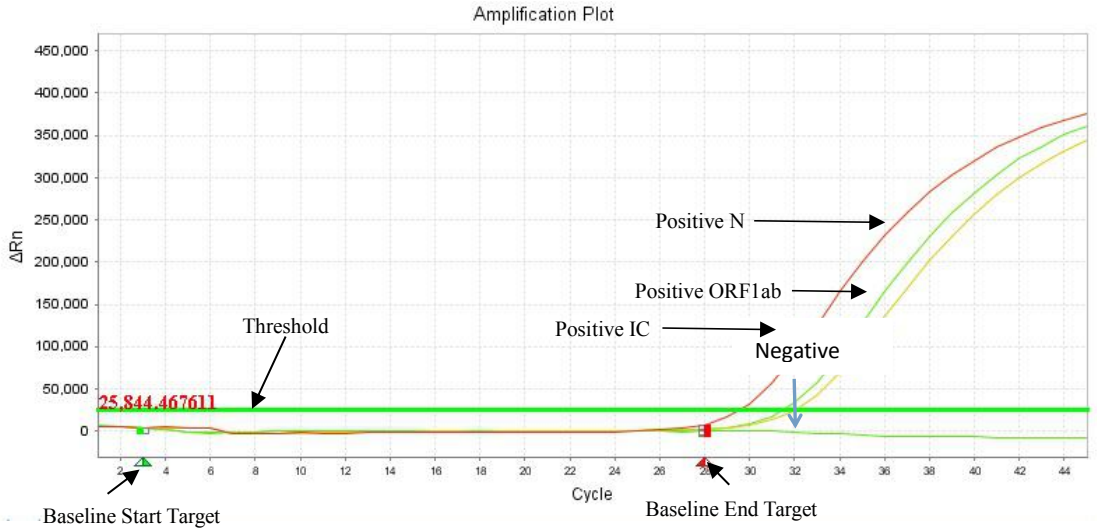
steps		temperature	time	cycles
1	UDG enzyme action	37°C	2 min	1
2	RNA denaturation	90°C	30 sec	1
3	RNA reverse transcription	61°C	15 min	1
4	Denaturation	95°C	3 sec	45
	Annealing, fluorescence signal gathering	60°C	10 sec	
Remarks: when using ABI 7500 series amplification instrument , time for step 4 should be set as 30sec.,and choose "None" in ROX dye correction settings.				

【Result analysis】

- Baseline setting: automatic optimization of the instrument for BIO-RAD CFX96. Set manually for ABI7500: Open Analysis Plot → Plot Setting SELECT Graph Type, Linear → Options SELECT Target, N, SET manually Threshold and Baseline, Baseline Start Target and Baseline End Target to

3~8Cycle and 24~30Cycle → Target SELECT ORF1ab and IC, SET same as above.

- Threshold setting: automatically by the instrument, or adjust manually according to the baseline that just exceeded the highest point of the amplification curve of the negative control,General Manually set the threshold line at the about 1/10th of the End point fluorescence value.
- Analyze the curves of SARS-CoV-2 and internal control respectively.



【Test Run Criteria】

- Negative control: N/A , or undetermined and internal control ct≤35.
- Positive control: Ct ≤ 40,the internal control results are not considered.
- Both criteria should be met, otherwise the test run is invalid.

【Result Interpretation】

- If **ORF1ab** and **N** are both positive (Ct≤40), **SARS-COV-2** has been detected.
- If only **ORF1ab** or **N** is positive, repeat the testing. If after repeating any of the two targets is still positive, **SARS-COV-2** has been detected.
- Repeat if the Ct value is 40<Ct<45 and interpreted according to re-test results. (If the re-test still has an amplification curve and Ct < 45, the result is interpreted as a positive)
- If the result is negative and the Ct of the IC is ≤35, the test result should be reported as negative for **SARS-COV-2**, otherwise the test run is invalid and the testing should be repeated.
- If the test result is positive, the internal control results are not considered.

【Limitations】

- This kit is only used for the qualitative detection of SARS-CoV-2 RNA.
- Do not rely solely on the results of this kit for a diagnosis. For a final diagnosis, the results of this kit should be considered in conjunction with the patient's symptoms, physical signs, medical history, other laboratory examinations and reactions to the treatments.
- The primer probe have been designed to detect the highly conservative regions of the ORFlab and N genes of the virus. However due to the high mutation rates of the RNA viruses, low possibility of mutation within the conservative regions still exists which may lead to false negative results with this kit.
- Improper sampling, transportation, storage and handling may cause errors in the results.
- The clinical lab should strictly follow the related clinical molecular diagnostic tests regulations and guidelines. Strictly follow the manual when carrying out the test.
- This kit is limited to the detection of throat swab, sputum, alveolar lavage fluid, tracheal aspirate samples.
- This kit is only applicable for the specified instruments.

【Performance Characteristics】

- The performance evaluation results of the kit have been conducted on the Applied Biosystem® 7500 Real-Time PCR system and Bio-Rad CFX 96 instruments.
- Sensitivity: The analytical sensitivity was determined by spiking negative oropharyngeal samples with RNA template. 4 control levels were prepared. The testing results demonstrated that the analytical sensitivity of the kit is 50 copies/ml of SARS-CoV-2 RNA (CI ≥95%). The analytical sensitivity was further validated by testing 10 replicates of 50 copies/ml control level on 3 kit lots.

Establishing of the analytical sensitivity of the kit

Level	copies/ml	ORF1ab Gene			N Gene		
		Detection	Mean Cq	Cq SD	Detection	Mean Cq	Cq SD
1	100	100%	37.16	0.63	100%	35.14	0.51
2	50	95%	38.4	0.88	100%	35.91	0.51
3	25	85%	38.29	0.59	85%	38.65	1.11
4	10	75%	40.52	0.8	60%	39.23	1

Analytical sensitivity validation
results on Applied Biosystem® 7500 Real-Time PCR system, Lot-1,2,3

Sample	Lot-1					
	ORF1ab Gene			N Gene		
	% Detection	Mean Cq	Cq SD	% Detection	Mean Cq	Cq SD
1	100%	35.74	0.54	100%	34.44	0.32
2	100%	35.95	0.51	100%	34.17	0.37
3	100%	36.18	0.51	100%	34.23	0.37
4	100%	36.06	0.54	100%	34.76	0.31
5	100%	35.57	0.54	100%	34.29	0.32
6	100%	36.20	0.52	100%	34.26	0.37
7	100%	35.80	0.55	100%	34.52	0.32
8	100%	36.04	0.54	100%	34.72	0.31
9	100%	36.05	0.51	100%	34.38	0.37
10	100%	36.12	0.54	100%	34.82	0.31

Sample	Lot-2					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.38	0.53	100%	34.52	0.37
2	100%	35.86	0.57	100%	34.28	0.36
3	100%	36.07	0.58	100%	34.33	0.36
4	100%	35.69	0.53	100%	34.80	0.36
5	100%	35.22	0.53	100%	34.37	0.38
6	100%	36.11	0.54	100%	34.34	0.36
7	100%	35.44	0.53	100%	34.58	0.36

8	100%	35.67	0.53	100%	34.77	0.36
9	100%	35.95	0.58	100%	34.48	0.36
10	100%	35.75	0.53	100%	34.86	0.36

Sample	Lot-3					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.65	0.45	100%	34.51	0.51
2	100%	36.12	0.38	100%	34.34	0.55
3	100%	36.36	0.38	100%	34.40	0.55
4	100%	35.97	0.45	100%	34.82	0.49
5	100%	35.48	0.45	100%	34.35	0.52
6	100%	36.34	0.40	100%	34.46	0.53
7	100%	35.71	0.45	100%	34.59	0.51
8	100%	35.95	0.45	100%	34.79	0.49
9	100%	36.23	0.38	100%	34.56	0.53
10	100%	36.03	0.46	100%	34.89	0.49

Analytical sensitivity validation
results of CFX 96 (Bio-Rad®) Real-Time PCR system, Lot-1,2,3

Sample	Lot-1					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.74	0.54	100%	34.44	0.32
2	100%	35.95	0.51	100%	34.17	0.37
3	100%	36.18	0.51	100%	34.23	0.37
4	100%	36.06	0.54	100%	34.76	0.31
5	100%	35.57	0.54	100%	34.29	0.32
6	100%	36.20	0.52	100%	34.26	0.37
7	100%	35.80	0.55	100%	34.52	0.32
8	100%	36.04	0.54	100%	34.72	0.31
9	100%	36.05	0.51	100%	34.38	0.37
10	100%	36.12	0.54	100%	34.82	0.31

Sample	Lot-2					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.65	0.53	100%	34.51	0.37
2	100%	36.12	0.57	100%	34.34	0.36

3	100%	36.36	0.58	100%	34.40	0.36
4	100%	35.97	0.53	100%	34.82	0.36
5	100%	35.48	0.53	100%	34.35	0.38
6	100%	36.34	0.54	100%	34.46	0.36
7	100%	35.71	0.53	100%	34.59	0.36
8	100%	35.95	0.53	100%	34.79	0.36
9	100%	36.23	0.58	100%	34.56	0.36
10	100%	36.03	0.53	100%	34.89	0.36

Sample	Lot-3					
	ORF1ab Gene			N Gene		
	% Replicate Detection	Mean Cq	Cq Standard Deviation	% Replicate Detection	Mean Cq	Cq Standard Deviation
1	100%	35.65	0.53	100%	34.51	0.37
2	100%	36.12	0.57	100%	34.34	0.36
3	100%	36.36	0.58	100%	34.40	0.36
4	100%	35.97	0.53	100%	34.82	0.36
5	100%	35.48	0.53	100%	34.35	0.38
6	100%	36.34	0.54	100%	34.46	0.36
7	100%	35.71	0.53	100%	34.59	0.36
8	100%	35.95	0.53	100%	34.79	0.36
9	100%	36.23	0.58	100%	34.56	0.36
10	100%	36.03	0.53	100%	34.89	0.36

- **Analytical Specificity:** No cross reactivity has been observed after the testing of samples from individuals infected with influenza and parainfluenza virus,H1N1 ,HN1(2009), H3N2, H5N1, H7N9, EBV, CMV, Adenovirus1,2,3,4,5,7, RSV A,RSV B, Rotavirus, Norovirus, Mycoplasma pneumoniae and Chlamydia.
- **Precision:** the precision of the kit was evaluated by testing of RNA-spiked negative oropharyngeal samples. 2 laboratory technicians tested 3 batches of kits with 10 replicates of 3 different concentrations (positive 5000 copies/ml, borderline 50 copies/ml and negative 0 copies/ml) for 5 days. The study was conducted using ABI7500and CFX-9 PCR instruments. The results from the study demonstrated very good precision of the kit with CV<5%.

Technician -1, ABI7500

Days	Lot nCoVP20200101			Lot nCoVP20200102			Lot nCoVP20200103		
	POS	Border	NEG	POS	Border	NEG	POS	Border	NEG
1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Technician- 1,CFX-96

Days	Lot nCoVP20200101			Lot nCoVP20200102			Lot nCoVP20200103		
	POS	Border	NEG	POS	Border	NEG	POS	Border	NEG
1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Technician-2, ABI7500

Days	Lot nCoVP20200101			Lot nCoVP20200102			Lot nCoVP20200103		
	POS	Border	NEG	POS	Border	NEG	POS	Border	NEG
1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Technicians 2,CFX-96

Days	Lot nCoVP20200101			Lot nCoVP20200102			Lot nCoVP20200103		
	POS	Border	NEG	POS	Border	NEG	POS	Border	NEG
1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

- Accuracy:Oropharyngeal and nasopharyngeal from healthy individuals,100 each were tested on 3 lots to validate the specificity of the kit and human DNA cross-reactivity. The results from the study given below indicate very good specificity of the kit with no cross- reactivity due to presence of human DNA.

Testing of 3 lots on negative samples

RT-PCR	Specimen	Type nCoV	Lot nCoVP20200101 (NEG/Total)	Lot nCoVP20200102 (NEG/Total)	Lot nCoVP20200103 (NEG/Total)
ABI7500	Nasopharyngeal	NEG	100/100	100/100	100/100
	Oropharyngeal	NEG	100/100	100/100	100/100
CFX-96	Nasopharyngeal	NEG	100/100	100/100	100/100
	Oropharyngeal	NEG	100/100	100/100	100/100

- **Clinical studies:** This kit has been evaluated at Beijing's Center for Disease Control and Prevention, and at the Institute of Microbial Epidemiology, Academy of Military Medical Sciences, Beijing, China. Total of 194 samples from the patients who were clinically confirmed for COVID-19 infection and 118 SARS-CoV-2 RNA negative samples were tested. The positive detection rate of Wantai SARS-CoV-2 RT-PCR was 183/194=94.33% (95% CI 90.08%－97.14%), and the specificity of the kit was 118/118=100% (95% CI 96.92%－100.00%). When the performance of the kit was compared against other commercially available nucleic acid detection kit (China CFDA-EUA approved assay), Wantai SARS-CoV-2 RT-PCR demonstrated positive and negative agreements

with the comparison tests of 171/172=99.42% (95% CI 96.80%－99.88%) and 134/160=83.75% (95% CI 77.10－89.10%) respectively. In total, 26 clinically confirmed samples were positive with Wantai but negative with the comparison test, and one clinically confirmed sample was negative with Wantai but positive with the comparison test. In summary, within the scope of the this study, Wantai SARS-CoV-2 RT-PCR demonstrated better sensitivity than the comparison test, the test also demonstrated very high detection rate in samples from patients clinically confirmed for COVID-19.

- **Interfering substances:** The following substances do not affect test results. 0.2mg/l of beclomethasone, 0.15mg/l of dexamethasone, 12mg/L of triamcinolone, 0.4mg/l of budesonide, 0.05mg/L of mometasone, 0.5mg/l of fluticasone, 75mg/L of benzocaine, 5mg/L of zanamivir, 37.5mg/l of oseltamivir, 75mg/L of tobramycin, 50mg/L of amantadine, 75mg/L of sulfur, 150mg/L of thryallis, 50mg/L of methyljinamine, 0.125mg/l of adrenaline, 25mg/L of menthol, 0.05% of hydroxymethazoline, 500mg/L of flunisolide, 500mg/L of mupirocin, 400mg/L of purified mucin, 200μl of hemolytic blood.

【Considerations】

- This product is for in-vitro diagnostic use only. Read the instructions for use before using the kit.
- This kit should be used only by qualified laboratory professionals.
- Reagents from different batches are not interchangeable.
- Do not mix with reagents from other commercially available kits.
- Samples and disposables left after the testing are potentially infectious. Discard used pipette tips into the biological waste container containing disinfectant before disposing. After testing, in order to avoid lab contamination, use 75% ethanol to clean the work station. Disinfect with an ultraviolet lamp. Handling should follow the established guidelines for biosafety of microbiological biomedical laboratories, management of medical waste, and other related normative guidelines.
- Lab management should strictly follow established national molecular biology laboratory and clinical gene amplification laboratory management standards. Laboratory personnel who perform the test must undergo professional training: The test should be carried in different areas (kit preparation area, sample preparation area, amplification and analysis area.) Each phase of the test uses special-purpose instruments and equipment. Cross-use of equipment from different phases and areas is prohibited. Staff and air circulation should be strictly regulated. Avoid cross-contamination as much as possible. Test disposable items should be thoroughly disinfected and inspected in order to avoid contamination or false negative results caused by amplification reaction inhibitor.
- After nucleic acid extraction, immediately take off the 8 sleeve groove tubes from the instrument. The extracting plate should be sealed after use in order to avoid aerosol pollution.

【CE Marking Symbols】

IVD	**In Vitro Diagnostic Medical Device**		**<-15°C Storage Conditions**
	Use By	LOT	**Batch**
	Content Sufficient For <n> Tests		**Instructions For Use**
CE	**CE Marking – IVDD 98/79/EC**	EC REP	**EU Authorized Representative**
REF	**Catalog Number**		**Manufacturer**

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CE

Version: V. 2020-01 [Eng.]
Issuing Date: March 4, 2020
Number of revision: Revision 1 (03/07)