hepatitisA@ceeramTools™

Technical note

1. Introduction

Developed by CEERAM SAS, this kit allows the detection of hepatitis A virus (HAV) by real time RT-PCR, in all types of environmental (water, wasterwater, seawater, sludge, air, surface…) and food samples (shellfish, fruits, vegetables, processed foods…).
Hepatitis A virus is a non-enveloped single strand RNA virus. The delay of incubation is around one month. The severity of the clinical signs increases with the going age of asymptomatic case in the young children (< 6 years old) with cases of acute or fulminant hepatitis. The transmission via the fecal-oral route is the most usual. Food samples can be contaminated via food handlers or by the environment. Due to their structural properties, Hepatitis A virus can resist to different physical, chemical treatment and food processes. Adsorbed on particles, they can survive in different kind of environment for a long period. The consummation of contaminated food can lead to human outbreak.
There is no specific curative treatment but a vaccine is available.
The diagnostic by cellular culture is difficult. Detection by real time RT-PCR, after extraction and purification of the viral RNA, is a method of choice taking into account its speed and its sensitivity. The detection limit varies from 1 to 10 copies depending of the quality of the analyzed nucleic acid.

Hepatitis A virus detection by real-time RT-PCR is the subject of an international patent for which CEERAM has an exclusive license.

Patent # PCT/EP2007/055402
(http://www.ceeram.com/licences-brevets-ceeram.html)

2. Kit contents

Cap color	Tube name	Volume
	HAV/IPC* Master Mix	950 µL tube (48 reactions)
	RT-PCR enzyme mix	55 µL tube (48 reactions)
	HAV Positive control	65 µL tube
	Negative control	250 µL tube

***IPC : Internal Positive Control**

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✓**HAV/IPC Master Mix :**

- Hepatitis A specific nucleotides set with FAM™ probe
- IPC specific nucleotides set with VIC® - TAMRA™ probe
- ROX fluorescence reference

Primers and probes used in this kit are in conformity regarding the ISO/TS 15216-1 & 2

3. Storage

Upon receiving the kit, make sure the case is frozen and sealed. Do not use the product if it is thawed or damaged. If this is the case, contact support@ceeramtools.com.

The kit must be stored at -20°C and protected from sunlight. Under these conditions, the kit can be used until the expiration date marked on both the attached certificate of conformity and on the labels. An inappropriate storage can affect reagents quality.

4. Using the kit / Recommendations

Minimize freeze-thaw cycles for all reagents in the kit. Do not exceed 3 cycles of freezing and thawing, as this may reduce the performance of the kit. Make aliquots if necessary, except for the "RT-PCR enzyme mix" tube.

When using, completely thaw the reagents at room temperature around 15 to 25°C, except for the "RT-PCR enzyme mix" tube.
Do not mix reagents from different kits.
Do not mix reagents from different batches.
Return all reagents to a -20°C environment immediately after use.

Samples : we recommend to **purify nucleic acids extracts** before using the kit (not included).
To profit from advises concerning sample processing and nucleic acids extraction and purification methods, please contact our experts : support@ceeramtools.com

5. User-supplied materials (not included)

✓ **Materials**

- Microplates or 0,2 mL tubes for real time RT-PCR systems
- Nuclease free pipettors, tips and tubes

✓ **Real time thermal cycler**

The *hepatitisA@ceeramTools* kit has been tested and is compatible on Applied Biosystems ABI Prism® 7300, 7500, StepOne™ and StepOnePlus™, PikoReal™, Stratagene Mx3000P® and Mx4000® and Roche Lightcycler®. Use a calibrated thermocycler for the fluorescence channel reading FAM™, TAMRA™, VIC® and ROX™ or equivalent. If not, make a color calibration on the instrument. To know the compatibility of your thermocycler with this product please contact *support@ceeramtools.com*.

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Troubleshooting

	Observations	Possible causes	Actions
IPC	No **IPC**[1] signal **and** no **target-specific**[2] signal in all wells	PCR inhibited	Repeat the assay in advised conditions diluting the sample 1:10 Use a purification kit adapted to your sample then repeat the assay in advised conditions
		Damaged reagents	Repeat the assay using properly stored reagents Avoid freeze/thaw cycles
		Pipetting errors	Repeat the assay in advised conditions. Make sure your equipment is clean and correctly calibrated
	No **IPC** signal **but** **target-specific** signal in target wells	High copy number of target resulting in preferential amplification of target-specific nucleic acids	Check IPC signal in the negative control well : **- if the signal is present :** **Optional verification :** repeat the assay in advised conditions diluting the sample 1:10 **- if the signal is absent :** repeat the assay in advised conditions using properly stored reagents
Positive Control	No signal **target-specific** signal **but** **IPC** signal in the positive control well	Damaged reagents **or** Pipetting error	Repeat the assay in advised conditions. Make sure to pipette positive control in the positive control well Repeat the assay in advised conditions using properly stored reagents Avoid freeze/thaw cycles If the positive control well is still show contamination, repeat the assay in advised conditions using a new kit
	No **IPC** signal **and** no **target-specific** signal in the positive control well	Damaged reagents	Repeat the assay in advised conditions using properly stored reagents Avoid freeze/thaw cycles
Negative Control	**Target-specific** signal in the negative control well	Contamination by samples or positive control	Repeat the assay in advised conditions If contamination persist, repeat the assay using fresh aliquots. Make sure your material is clean and correctly calibrated If contamination persist, repeat the assay in advised conditions using a new kit

[1]**IPC Signal :** results of amplification of an internal amplification control distinguishable from the sought target, allow the validation of the PCR reaction and make it possible to measure matrix inhibition.
[2]**Target specific signal :** results of amplification of a sample or a positive control and correspond to the sought target.

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Technical support

For technical support, please contact our experts: support@ceeramtools.com

Supplementary Information

Label License Statement

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,804,375, 5,538,848, 5,723,591, 5,876,930, 6,030,787 , 6,258,569, 6,127,155 5,677,152 (claims 1 to 23 only) and 5,773,258 (claims 1 and 6 only). The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product solely in Food and Water Safety Testing Applications, including reporting results of purchaser's activities for a fee or other commercial consideration, and also for the purchaser's own internal research. No right under any other patent claim is conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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