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LightCycler® TaqMan® Master

Version 08

Content version: September 2011

Ready-to-use hot start reaction mix for PCR on the LightCycler® Carousel-Based System* using Hydrolysis TaqMan® Probes

Cat. No. 04 535 286 001	Kit for 96 reactions
Cat. No. 04 735 536 001	Kit for 480 reactions

Store the kit at −15 to −25°C

*LightCycler® 2.0 Instrument, LightCycler® 1.5 Instrument or a lower instrument version!

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Table of Contents

PROTOCOL

1. What this Product Does

Number of Tests

The kit is designed for:

- Cat.No.04 535 286 001: 96 reactions, with a reaction volume of 20 µl each
- Cat.No. 04 735 536 001: 480 reactions, with a reaction volume of 20 µl each

Kit Contents

Vial/Cap	Label	Contents/Function a) Cat. No. 04 535 286 001 (96 reactions) b) Cat. No. 04 735 536 001 (480 reactions)
1a white cap	Enzyme	a) 1× vial 1a, 3× vial 1b, for 3× 128 µl Master Mix (5x conc.) b) 5× vial 1a, 15× vial 1b, for 15× 128 µl Master Mix (5× conc.) - Ready-to-use hot start PCR reaction mix (after pipetting 10 µl from vial 1a into one vial 1b) - Contains FastStart Taq DNA Polymerase, reaction buffer, $MgCl_2$ and dNTP mix (with dUTP instead of dTTP)
1b red cap	Reaction Mix	
2 colorless cap	Water, PCR grade	a) 2× 1 ml b) 7× 1 ml - to adjust the final reaction volume

Storage and Stability

- Store the kit at −15 to −25°C until the expiration date printed on the label.
- The kit is shipped on dry ice.
- Once the kit is opened, store the kit components as described in the following table:

Vial	Label	Storage
1a white cap	Enzyme	- Store at −15 to −25°C. - **Avoid repeated freezing and thawing!** - **Keep vial 1b away from light!**
1b red cap	Reaction Mix	
1 red cap (after addition of 1a to 1b)	Master Mix	- Store at −15 to −25°C for a maximum of 3 months. - After thawing, store at +2 to +8°C for a maximum of one week. - **Avoid repeated freezing and thawing!**
2 colorless cap	Water, PCR-grade	- Store at −15 to −25°C.

Additional Equipment and Reagents Required

Refer to the list below for additional reagents and equipment required to perform PCR reactions with the LightCycler® TaqMan® Master using the LightCycler® Carousel-Based System:

- LightCycler® Carousel-Based System* (LightCycler® 2.0 Instrument*, LightCycler® 1.5 Instrument*, or a lower instrument version)
- LightCycler® Capillaries* (20 µl)
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes

  The LightCycler® Carousel-Based System provides adapters that allow LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.

or

LC Carousel Centrifuge 2.0* for use with the LightCycler® 2.0 Sample Carousel (optional)

If you use a LightCycler® Instrument version below 2.0, you additionally need the LC Carousel Centrifuge 2.0 Bucket 2.1*. To adapt the LightCycler® 2.0 Sample Carousel to the former LC Carousel Centrifuge, you need the LC Carousel Centrifuge 2.0 Rotor Set*.

- PCR template (genomic DNA or cDNA)
- PCR primers
- Hydrolysis probe
- LightCycler® Uracil-DNA Glycosylase* (optional‡)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing master mixes and dilutions

‡For prevention of carry-over contamination; see Related Procedures section for details.

**available from Roche Applied Science; see Ordering Information for details*

Application

The LightCycler® TaqMan® Master is designed for life science research. In combination with the LightCycler® Carousel-Based System, the kit enables high sensitive detection and quantification of defined DNA sequences (with suitable PCR primers and detection probes). It can also be used to detect and quantify defined RNA sequences in a two-step RT-PCR (with additional reagents for reverse transcription).

To prevent false-positives due to contamination with amplified DNA, the kit can be used with LightCycler® Uracil-DNA Glycosylase.

In principle, the kit can be used for the amplification and detection of any DNA or cDNA target. However, you would have to adapt each amplification protocol to the reaction conditions of the LightCycler® Carousel-Based System and design a pair of specific PCR primers and a Hydrolysis probe for each target.

⚠ The amplicon size should not exceed 1 kb in length. For optimum results, select a product length of 700 bp or less.

⚠ The performance of the kit described in this Instruction Manual is warranted only when it is used with the LightCycler® Carousel-Based System.

Assay Time

Procedure	Time
PCR Set-up	20 min
LightCycler® Carousel-Based System PCR run	40 min
Total assay time	**60 min**

2. How to Use this Product

2.1 Before You Begin

Sample Material

Use any template DNA (*e.g.,* genomic or plasmid DNA, cDNA) suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids use:

- either the MagNA Pure LC Instrument and a dedicated MagNA Pure LC reagent kit (for automated isolation) or
- a High Pure Nucleic Acid Isolation Kit (for manual isolation).

For details see the Roche Applied Science catalogue or the website: **www.roche-applied-science.com**

If you are using a non-purified cDNA sample from reverse transcription, especially if it contains high background concentrations of RNA and oligonucleotides, you can improve your results by using 2 µl or less of sample in the reaction.

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water (vial 2, colorless cap).

Primers

Use PCR primers at a final concentration of 0.1 – 1 µM. The recommended starting concentration is 0.5 µM in each case.

Hydrolysis Probes

Use Hydrolysis probes at a final concentration of 0.05 – 0.1 µM. In some cases the fluorescence signal may be increased with a higher probe concentration of up to 0.2 µM.

$MgCl_2$

All components in the reaction mix of the LightCycler® TaqMan® Master are optimized for almost all primer combinations. You do not need to add additional $MgCl_2$ to the mix to produce an efficient and specific PCR.

2.2 Procedure

LightCycler® Carousel-Based System Protocol

The following procedure is optimized for use with the LightCycler® Carousel-Based System.

Program the LightCycler® Instrument before preparing the reaction mixes. A LightCycler® Carousel-Based System protocol that uses LightCycler® TaqMan® Master contains the following programs:

- **Pre-Incubation** (activation of FastStart DNA polymerase and denaturation of the DNA)
- **Amplification** of the target DNA
- **Cooling** the rotor and thermal chamber

For details on how to program the experimental protocol, see the LightCycler® 2.0 Instrument Operator's Manual.

⚠ Set all other protocol parameters not listed in the tables below to '0'.

The following table shows the PCR parameters that must be programmed for a LightCycler® Carousel-Based System PCR run with the LightCycler® TaqMan® Master.

Analysis Mode	Cycles	Segment	Target Temperature [1]	Hold Time	Acquisition Mode
Pre-Incubation					
None	1		95°C	10 min[2]	none
Amplification					
Quantification	45[3]	Denaturation	95°C	10 s	none
		Annealing	primer dependent [4]	20 – 40 s[5]	none
		Extension	72°C	1 s	single
Cooling					
None	1		40°C	30 s	none

[1] Temperature Transition Rate/Slope is 20°C/sec.

[2] If high polymerase activity is needed in early cycles, you can sometimes improve results by extending the pre-incubation to 15 min.

[3] 45 cycles are suitable for most of the assays. Nevertheless, for a well optimized assay with steep amplification curves and early crossing points, even with low target concentrations, 40 cycles should be sufficient (resulting in a reduced assay time!).

[4] For initial experiments, set the target temperature 5°C below the calculated primer melting temperatures.

[5] For higher precision in target quantification experiments, in some cases it can be advantageous to choose relatively long annealing times, up to 45 s when amplifying sequences > 500 bp.

Fluorescence and Run Setup Parameters

Parameter	Setting	
All LightCycler® Software Versions		
Seek Temperature	30°C	
LightCycler® Software prior to Version 3.5		
Display Mode	fluorescence channel F1(for using a FAM-labeled hydrolysis probe)	
Fluorescence Gains	**Fluorimeter**	**Gain Value**
	Channel 1 (F1)	1
	Channel 2 (F2)	15
	Channel 3 (F3)	30
LightCycler® Software Version 3.5		
Display Mode	fluorescence channel F1(for using a FAM-labeled hydrolysis probe)	
Fluorescence Gains	Not required In data created with LightCycler® Software Version 3.5, all fluorescence values are normalized to a fluorescence gain of "1". This produces a different scale on the Y-axis than that obtained with previous LightCycler® Software versions. This difference does not affect the crossing points or any calculated concentrations obtained.	

Parameter	Setting
LightCycler® Software Version 4	
Default Channel	Depending on the emission maximum of the fluorescent reporter dye select the next detection channel up.
Fluorescence Gains	Not required
"Max. Seek Pos"	Enter the number of sample positions the instrument should look for.
"Instrument Type"	▪ "6 Ch.": for LightCycler® 2.0 Instrument (selected by default) ▪ "3 Ch.": for LightCycler® 1.5 Instrument and lower instrument versions.
"Capillary Size"	Select "20 µl" as the capillary size for the experiment. ⚠ For the "6 Ch." instrument type only.

Preparation of the Master Mix

Prepare the 5× conc. Master Mix as described below.

❶	Thaw one vial of "Reaction Mix" (vial 1b, red cap).
❷	Briefly centrifuge one vial 1a ("Enzyme", white cap) and one thawed vial 1b ("Reaction Mix", red cap, from Step1).
❸	Pipet 10 µl from vial 1a (white cap) into vial 1b (red cap). 🔍 Each vial 1a contains enough enzyme for three vials of Reaction Mix.
❹	Mix gently by pipetting up and down. Do not vortex.
❺	Re-label vial 1b (red cap) with the new label (vial 1: Master Mix) provided with the kit.

🔍 The volume of the resulting Master Mix (5× conc.) is sufficient for 32 reactions with a final reaction volume of 20 µl in each case.

Preparation of the PCR Mix

Proceed as described below for a 20 µl standard reaction.

The protocol is designed for a final reaction volume of 20 µl. For volumes < 20 µl, the reaction and cycle conditions must be optimized.

⚠ Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.

1. Depending on the total number of reactions, place the required number of LightCycler® Capillaries in precooled centrifuge adapters or in a LightCycler® Sample Carousel in a precooled LightCycler® Centrifuge Bucket.
2. Prepare a 10x conc. solution that contains PCR primers and Hydrolysis probe.
3. Thaw the LightCycler® TaqMan® Master, 5× conc. (vial 1, red cap), mix gently and store on ice.
4. In a 1.5 ml reaction tube on ice, prepare one reaction by adding the following components in the order listed below, then mix gently by pipetting up and down:

Component	Volume
Water, PCR-grade (vial 2, colorless cap)	9 µl
Primers/Probe, 10× conc.	2 µl
Master Mix, 5× conc. (vial 1)	4 µl
Final volume	**15 µl**

   To prepare the PCR Mix for more than one reaction, multiply the amount in the "Volume" column above by the number of reactions to be run plus one additional reaction.

5. ▪ Pipet 15 µl PCR mix into each precooled LightCycler® Capillary.
   ▪ Add 5 µl of the DNA template.

   Use up to 500 ng complex genomic DNA or 10^1 – 10^{10} copies of plasmid DNA.

6. ▪ Seal each capillary with a stopper and place the adapters (containing the capillaries) in a standard benchtop microcentrifuge.

   ⚠ Place the centrifuge adapters in a balanced arrangement in the centrifuge.

   ▪ Centrifuge at 700 × *g* for 5 s (3,000 rpm in a standard benchtop microcentrifuge). Alternatively, use the LC Carousel Centrifuge for spinning the capillaries.
7. Transfer the capillaries to the sample carousel of the LightCycler® Instrument.
8. Cycle the samples as described above.

2.3 Related Procedures

Prevention of Carry-Over Contamination

Uracil DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination in PCR. This carry-over prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the Master Mix in this kit) into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template.

⚠ Since your target DNA template contains thymidine rather than uridine it is not affected by this procedure.

⚠ Refer to the package insert of LightCycler® Uracil-DNA Glycosylase for details on application.

Two-step RT-PCR

The LightCycler® TaqMan® Master can also be used to perform two-step RT-PCR.

In two-step RT-PCR, the reverse transcription of RNA into cDNA is separated from the other reaction steps and is performed outside the LightCycler® Carousel-Based System. Subsequent amplification and online monitoring is performed according to the LightCycler® Carousel-Based System standard procedure, using cDNA as starting sample material.

One of the following reagents is required for reverse transcription of RNA into cDNA (see Ordering Information for details):

- Transcriptor Reverse Transcriptase
- Transcriptor First Strand cDNA Synthesis Kit
- First Strand cDNA Synthesis Kit for RT-PCR (AMV)

Synthesis of cDNA is performed according to the detailed instructions provided with the cDNA synthesis reagent.

⚠ Do not use more than 8 µl of undiluted cDNA template per 20 µl final reaction volume because greater amounts may inhibit PCR. For initial experiments, we recommend running undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel to determine the optimum template amount.

3. Results

The following amplification curves were obtained with dilutions of human genomic DNA in 1:10 steps and a set of primers and a FAM/TAMRA-labeled probe, specific for a fragment of 442 bp of the cyclophilin A gene.

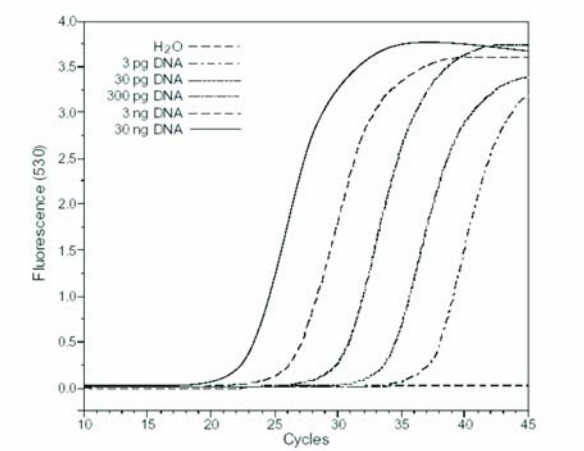

Fig. 1: Cyclophilin A amplification curves in the analysis module for absolute quantification of LightCycler® Software 4.0.

4. Troubleshooting

	Possible cause	Recommendation
Fluorescence curves reach maximum long before cycling is finalized.	Very high starting amount of nucleic acid.	Cycling can be finished by clicking the **End Program** button. The next program part will continue automatically.
	Number of cycles is too high.	Reduce the number of cycles in the protocol.
Log-linear phase of amplification just starts when cycle program finishes.	Number of cycles is too low.	▪ While cycling is still ongoing use the **Add 10 Cycles** button to increase the number of cycles. ▪ Increase the number of cycles in the protocol. ▪ Use higher amount of starting material. ▪ Optimize PCR conditions (primer/probe design, protocol).
No amplification detectable.	Wrong channel has been chosen to display amplification on screen.	Check the channel chosen for display. (All data from all channels are always saved.)
	Inhibitory effects of the sample material due to insufficient purification.	▪ Try a 1 : 10 dilution of your sample. ▪ Isolate the nucleic acids of your sample material to ensure removal of inhibitory agents.
	FastStart DNA Polymerase is not sufficiently activated.	▪ Check whether PCR was started with 10 min pre-incubation step at 95°C. ▪ Check whether denaturation time in cycles was about 10 sec.
	Pipetting errors or omitted reagents.	▪ Check for missing or defective reagents.
	Amplicon length is > 1 kb.	Do not use primers for amplicons > 1 kb. Amplification efficiency is reduced with amplicons of this size. Optimal results are obtained for amplicons < 700 bp.
	Difficult template *e.g.,* unusual GC-rich sequence.	▪ Optimize temperatures and times in the amplification cycles. ▪ Optimize primer/probe sequences. ▪ Repeat PCR with increasing content of DMSO, up to 10 % final concentration.
Fluorescence intensity varies.	Some of the reagent is still in the upper vessel of the capillary, or an air bubble is trapped in the capillary tip.	Ensure the centrifugation step was properly performed.
	Skin oils or dirt on the surface of the capillary.	Always wear gloves when handling the capillaries.

	Possible cause	Recommendation
	Pipetting inaccuracy	For compensation of slight variations in probe concentration divide the detection channel by channel 705. For example, when using a FAM-labeled hydrolysis probe, choose channel 533/705 for analysis (or F1/F3 in software version prior to version 4.0).
Fluorescence intensity is very low.	Low concentration or deterioration of dyes in the reaction mixtures due to unsuitable storage conditions.	▪ Protect dye-labeled reagents from light. ▪ Store the reagents at −15 to −25°C and avoid repeated freezing and thawing. ▪ After thawing, store the Master Mix at +2 to +8°C for a maximum of 1 week.
	Poor PCR efficiency due to non-optimized reaction conditions.	Check concentrations of reagents and LightCycler® Carousel-Based System protocol.
	Chosen gain settings are too low. (only with LightCycler® Software version < 3.3)	Use the **Real Time Fluorimeter** to find suitable gain settings. Change gain settings in the protocol and repeat the run. With LightCycler® Software version > 3.3 no gain setting is required.
Overflow of fluorescence intensity. (Only with LightCycler® Software version 3.3)	Unsuitable gain settings.	Gain settings cannot be corrected during or after a run. Before repeating the run, use the **Real Time Fluorimeter** to find suitable gain settings. With LightCycler® Software version > 3.3 no gain setting is required.
Negative control is positive.	Contamination.	▪ Exchange all critical solutions. ▪ Pipet on a clean bench. ▪ Close lid of the negative control reaction tube immediately after pipetting. ▪ Use UNG for prevention of contamination by amplicons.
High background	Very low fluorescence signals, therefore the background seems to be relatively high.	Follow general optimization strategies for LightCycler® Carousel-Based System PCR.
	Insufficient quality of probe.	Prepare a new solution of probe.

5. Additional Information on this Product

5.1 How this Product Works

LightCycler® TaqMan® Master is a ready-to-use reaction mix designed for the TaqMan® detection format using the LightCycler® Carousel-Based System.
It contains FastStart polymerase for a "Hot Start" PCR, which has been shown to significantly improve the specificity and sensitivity of PCR by minimizing the formation of non-specific amplification products [1, 2, 3, 4]. FastStart Taq DNA Polymerase is a chemically modified form of thermostable recombinant Taq DNA polymerase that is inactive at +15 to +25°C and below. The enzyme is active only at high temperatures, where primers no longer bind non-specifically. The enzyme is completely activated (by removal of blocking groups) in a single pre-incubation step (95°C, 10 minutes) before cycling begins. Activation does not require the extra handling steps typical of other “Hot Start” techniques.

The LightCycler® TaqMan® Master provides convenience, excellent performance, reproducibility, and minimal contamination risk. All you have to supply is PCR primers, a detection probe and your template DNA.

The reaction mix in this kit is optimized for a fixed $MgCl_2$ concentration, which works with nearly all primer combinations. You do not need to adjust the $MgCl_2$ concentration to amplify different sequences.

⚠ The performance of the kit described in this Instruction Manual is guaranteed only when it is used with the LightCycler® Carousel-Based System.

Test principle

Hydrolysis probe assays, also called TaqMan® assays, use a single probe containing two labels, a fluorescent reporter dye and a fluorescent quencher. While the probe is intact, the quencher is close to the reporter dye and suppresses the reporter fluorescence via fluorescence resonance energy transfer (FRET). When the probe is hybridized to the target sequence, the 5´-nuclease activity of the polymerase can cleave the hydrolysis probe, separating reporter and quencher. With a rising amount of target sequence during PCR, more probe is cleaved, and the fluorescence signal of the unquenched reporter dye increases.

⚠ As the principle of Hydrolysis probe assays is probe cleavage during PCR, Hydrolysis probes cannot be used to perform a melting curve analysis. In contrast, HybProbe probes which consist of two specially designed, sequence-specific oligonucleotide probes labeled with different dyes, are still intact at the end of amplification, and thus may be used in a subsequent melting curve experiment (*e.g.*, for mutation detection or SNP analysis).

References

1 Chou, Q *et al* (1992) *Nucleic Acid Res.* **20**,1717-1723.
2 Kellogg, D.E. *et al* (1994) *BioTechniques* **16**,1134-1137.
3 Birch, D.E. *et al* (1996) *Nature* **381**,445-446.
4 PCR Manual, Roche Diagnostics (1999) 2nd edition (1999) **2,** 52-58.

Quality Control

The LightCycler® TaqMan® Master is function tested using the LightCycler® Carousel-Based System.

6. Supplementary Information

6.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

Update of License Disclaimer

6.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Sites including:

- The LightCycler® Carousel-Based System family for real-time, online PCR: http://www.lightcycler-online.com
- The MagNA Pure family for automated nucleic acid isolation: http://www.roche-applied-science.com/sis/magnapure/
- Manual Nucleic Acid Isolation and Purification: http://www.roche-applied-science.com/napure/

	Product	Pack Size	Cat. No.
Instrument and Accessories	LightCycler® 2.0 Instrument	1 instrument plus accessories	03 351 414 001
	LightCycler® Capillaries (20 µl)	1 pack (8 boxes, each with 96 capillaries and stoppers)	11 909 339 001
	LC Carousel Centrifuge 2.0	1 rotor + 2 buckets	03 724 697 001
	LC Carousel Centrifuge 2.0 Bucket 2.1	1 bucket	03 724 689 001
	LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and bucket	03 709 507 001 (115 V) 03 709 582 001 (230 V)
Software	LightCycler® Software 4.1	1 software package	04 898 915 001
	LightCycler® Probe Design Software 2.0	1 software package	04 342 054 001
LightCycler® Kits for PCR	LightCycler® DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	12 015 102 001 12 158 825 001
	LightCycler® FastStart DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 003 248 001 12 239 272 001
	LightCycler® FastStart DNA Master^{PLUS} HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 515 575 001 03 515 567 001
	LightCycler® FastStart DNA Master^{PLUS} HybProbe, 100 µl Reactions	1 kit (384 reactions)	03 752 178 001
	LightCycler® DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	12 015 099 001 12 158 817 001
	LightCycler® FastStart DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
	LightCycler® FastStart DNA Master^{PLUS} SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 515 869 001 03 515 885 001
	LightCycler® FastStart DNA Master^{PLUS} SYBR Green I, 100 µl Reactions	1 kit (384 reactions)	03 752 186 001
Associated Kits and Reagents	LightCycler® Uracil-DNA Glycosylase	100 U (50 µl)	03 539 806 001

6.4 Disclaimer of License

NOTICE: This product may be subject to certain use restrictions. Before using this product please refer to the Online Technical Support page (http://technical-support.roche.com) and search under the product number or the product name, whether this product is subject to a license disclaimer containing use restrictions.

6.5 Trademarks

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6.6 Regulatory Disclaimer

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