For life science research use only. Not for use in diagnostic procedures. For *in vitro* use only.

LightMix®480HT *Scrapie Susceptibility* Mutation Detection

Cat.-No. 40-0297-96

Tricolor LightMix® for the detection of ovine prion protein (PRNP) gene mutations in the codons 136, 141, 154 and 171 in a single reaction using the LightCycler® 480 Instrument.

Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each – **store protected from light at room temperature (18-25°C), do NO T freeze!**

1. Set contents

1 Vial containing premixed and lyophilized primers and hybridization probes for 96 reactions

2. Experimental protocol

(A) Preparation of parameter-specific reagents (96 reactions):

One reagent vial contains all primers and probes to run 96 LightCycler® reactions.

Add 100 µl PCR-grade water to the reagent vial, mix the solution (vortex) and spin down.

►Use 1 µl **reagent** for a 20 µl PCR reaction.

This solution is stable for three days or longer if stored refrigerated at 4°C. Avoid prolonged exposu re to light.

(B) Preparation of the LightCycler® reaction mix

For use with the Roche FastStart kit		
Single reaction	**Component**	**final**
14.2 µl	water, PCR-grade (colorless cap, provided with the Roche FastStart or FastStart^{PLUS} kit)	--
2.0 µl	FastStart mix (vial 1 (red cap), combined from vials 1a and 1b, see Roche manual)	1x
0.8 µl	Mg^{2+} solution 25 mM (blue cap, provided with the Roche FastStart kit)	2 mM
1.0 µl	**reagent** mix (parameter specific reagents containing primers and probes, see **A**)	--
2.0 µl	sample or reference	--
20.0 µl	final volume	

Mix gently, spin down and transfer to a LightCycler® 480 Multiwell Plate.

3. Programming (LightCycler® 480 Instrument)

Block Type: 96 or 384

Detection Format: 450 – 500
483 – 533
450 – 610
450 – 640
483 – 670

Program:	**Denaturation**	**Cycling**			**Melting**			**Cooling**
Parameter								
Analysis Mode	None	Quantification			Melting Curves			None
Cycles	1	45			1			1
Segment	1	1	2	3	1	2	3	1
Target [°C]	95	95	60	72	95	40	75	40
Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:10	00:00:15	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [°C/s] ***96***	*4.4*	*4.4*	*2.2*	*4.4*	*4.4*	*1.5*	-	*1.5*
Ramp Rate [°C/s] ***384***	*4.6*	*4.6*	*2.4*	*4.6*	*4.6*	*2.0*	-	*2.0*
Acquisition Mode	None	None	Single	None	None	None	Continu.	None
Acquisitions [per °C]							1	

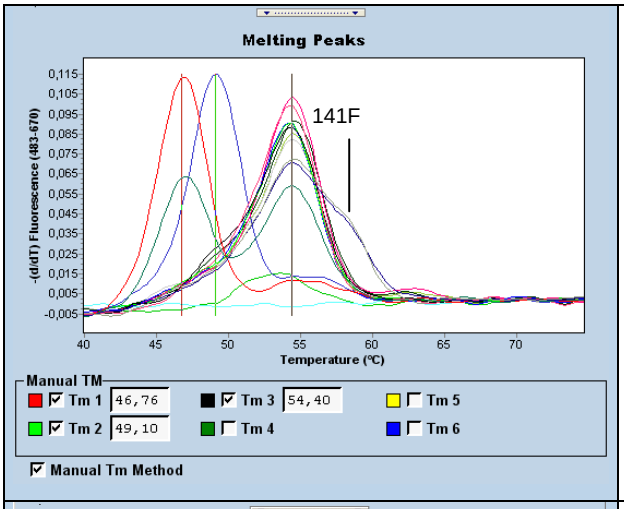
4. Data analysis

For data analysis use the melting curve analysis section.

Select Filter Combination 483-670 for the analysis of mutations in the codon 136 and 141 (**no color compensation** necessary).

Select Filter Combination 450-640 for the analysis of mutations in the codon 154 and Filter Combination 450-610 for the analysis of mutations in the codon 171. Switch the color compensation mode on. Channels to compensate: 450-500, 483-533, 450-610, 450-640, 483-670.

5. Sample data - typical results



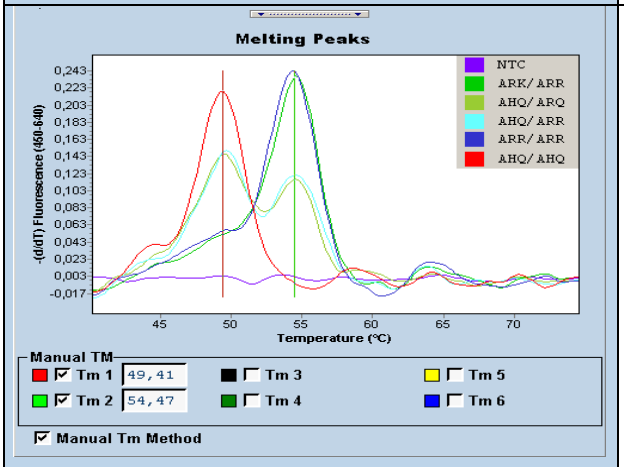
Left panel

Sample data for PRNP 136 and 141

Filter Combination 483-670

Position	Amino Acid	wt/mut	Melting temperature
136	A	Wildtype	54.4℃
136	V	Mutation	46.7℃
136	T	Mutation	49.1℃

Note: A specific signal of the genotype from Codon 141 is visible as 141F mutation (shoulder at 58℃), there is no specific wildtype signal visible.

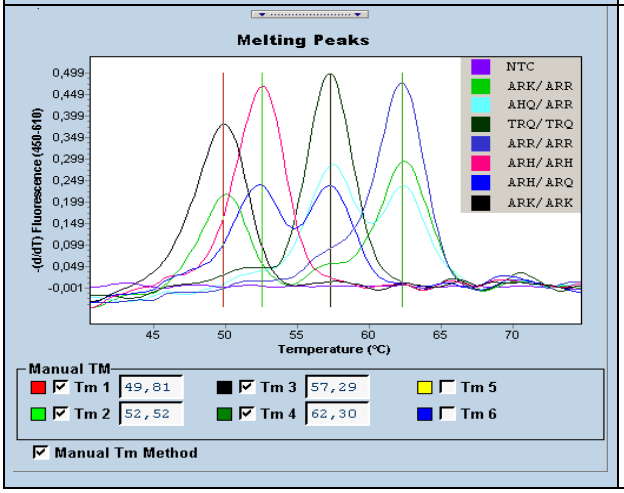


Left panel

Sample data for PRNP 154

Filter Combination 450-640

Position	Amino Acid	wt/mut	Melting temperature
154	R	Wildtype	54.5℃
154	H	Mutation	49.4℃



Left panel

Sample data for PRNP 171

Filter Combination 450-610

Position	Amino Acid	wt/mut	Melting temperature
171	Q	Wildtype	57.3℃
171	K	Mutation	49.8℃
171	H	Mutation	52.5℃
171	R	Mutation	62.3℃

Fig.1. Sample data for the *ov PRNP genotype* detection system
Note: Observed temperatures can differ due to buffer components (salt) coming from the extraction process and possibly due to different instrument settings. However, the temperature differences between the individual types are rather constant. Use known (pretyped) samples for calibration.

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Reverse primer was changed December 2006 based on the report of a deviation due to a SNP at codon 176; Version 317.

These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.
LightCycler® hybridization probes produced under license from Roche Diagnostics.


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