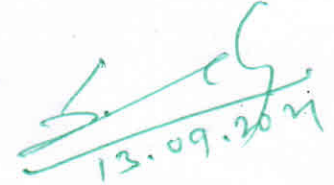


TO WHOMSOEVER IT MAY CONCERN

This is Certify that the following Extraction System INSTA NX MAG32 (LA1096) of Himedia Laboratories Pvt Ltd is Evaluated for Extraction of DNA From Different sample with Good quality and Yield. Real-Time PCR kits of HiMedia Laboratories Pvt Ltd. is compatible in HiMedia Insta Q Series and Applied Biosystems™ QuantStudio™ 5 and are giving satisfactory results for detection of Cow, Buffalo and Cattle Sex from different forensic samples.

1. MBPCR184: Hi-PCR® Cow Detection Kit
2. MBPCR185: Hi-PCR® Buffalo Detection Kit
3. MBPCR186: Hi-PCR® Cattle Sex Detection Kit
4. MBPCR210: Hi-PCR® Cow-Bufferalo Detection Kit


13.09.2021

Dr Anil Kumar Singh
In-Charge, DNA Unit
RFSL BHOPAL, MP


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Report of MBPCR184: Hi-PCR® Cow Detection Kit, MBPCR185: Hi-PCR® Buffalo Detection Kit & MBPCR186: Hi-PCR® Cattle Sex Detection Kit in HiMedia's Insta Q96® Plus & Applied Biosystems™ QuantStudio™ 5

1. The following samples were processed:
 - a. 6 blood samples
 - i. Buffalo Female
 - ii. Buffalo Male
 - iii. Bullock
 - iv. Cow Female Blood
 - v. Human blood
 - vi. Monkey blood
 - b. 5 Tissue samples
 - i. Buffalo meat
 - ii. Chicken meat
 - iii. Goat meat
 - iv. Human Tissue Formalin
 - v. Human Tissue Normal saline
 - c. 2 Milk samples
 - i. Milk as such processed as blood protocol
 - ii. Milk digested processed as tissue protocol
2. All 13 samples were processed by HiMedia's MB554MPF32200 HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32 in Insta NX® Mag32 system (LA1096).
3. All the 13 DNA samples were amplified for the following Real-Time PCR kits:
 - a. MBPCR184: Hi-PCR® Cow Detection Kit, Lot No: 0000493223
 - b. MBPCR185: Hi-PCR® Buffalo Detection Kit, Lot No: 0000493225
 - c. MBPCR210: Hi-PCR® Cow-Bufferalo Detection Kit, Lot No: 0000493227
 - d. MBPCR186: Hi-PCR® Cattle Sex Detection Kit, Lot No: 0000493226
4. Two Real-Time PCR machines were used:
 - a. HiMedia's Insta Q96® Plus
 - b. Applied Biosystem's QuantStudio 5
 - c. The following channels were used in the two Real-Time PCR machines

HiMedia's Insta Q96® Plus (LA1073)			
MBPCR184, Hi-PCR® Cow Detection Kit	MBPCR185, Hi-PCR® Buffalo Detection Kit	MBPCR210, Hi-PCR® Cow-Bufferalo Detection Kit	MBPCR186, Hi-PCR® Cattle Sex Detection Kit
FAM – Cow	FAM – Buffalo	FAM – Cow	FAM – X chromosome
JOE – Internal control	JOE – Internal control	JOE – Buffalo	HEX – Y chromosome
		Texas Red – Internal control	Texas Red – Internal control

Applied Biosystem's QuantStudio 5			
MBPCR184, Hi-PCR® Cow Detection Kit	MBPCR185, Hi-PCR® Buffalo Detection Kit	MBPCR210, Hi-PCR® Cow-Bufferalo Detection Kit	MBPCR186, Hi-PCR® Cattle Sex Detection Kit
FAM – Cow	FAM – Buffalo	FAM – Cow	FAM – X chromosome
VIC – Internal control	VIC – Internal control	VIC – Buffalo	VIC – Y chromosome
		ROX – Internal control	ROX – Internal control


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5. The following results were obtained for the four Real-Time PCR kits in the two machines

MBPCR184: Hi-PCR® Cow Detection Kit							
InstaQ 96 Plus (LA1073)				QuantStudio 5			
Sample ID	Target Name	Dye	Ct	Sample ID	Target Name	Dye	Ct
Cow Female Blood	Cow 184	FAM	17.02	Cow Female Blood	Cow 184	FAM	18.17
Cow Female Blood	IC 184	JOE	13.83	Cow Female Blood	IC 184	VIC	15.23
Bullock	Cow 184	FAM	17.4	Bullock	Cow 184	FAM	18.83
Bullock	IC 184	JOE	14.02	Bullock	IC 184	VIC	14.98
Buffalo meat	Cow 184	FAM	UD	Buffalo meat	Cow 184	FAM	UD
Buffalo meat	IC 184	JOE	14.01	Buffalo meat	IC 184	VIC	15
Buffalo Male	Cow 184	FAM	UD	Buffalo Male	Cow 184	FAM	UD
Buffalo Male	IC 184	JOE	13.8	Buffalo Male	IC 184	VIC	15.03
Goat meat	Cow 184	FAM	UD	Goat meat	Cow 184	FAM	UD
Goat meat	IC 184	JOE	13.49	Goat meat	IC 184	VIC	15
Buffalo Female	Cow 184	FAM	UD	Buffalo Female	Cow 184	FAM	UD
Buffalo Female	IC 184	JOE	13.81	Buffalo Female	IC 184	VIC	14.96
Chicken meat	Cow 184	FAM	UD	Chicken meat	Cow 184	FAM	UD
Chicken meat	IC 184	JOE	13.64	Chicken meat	IC 184	VIC	15.08
Milk digested	Cow 184	FAM	18.69	Milk digested	Cow 184	FAM	20.04
Milk digested	IC 184	JOE	13.76	Milk digested	IC 184	VIC	15.06
Monkey blood	Cow 184	FAM	UD	Monkey blood	Cow 184	FAM	UD
Monkey blood	IC 184	JOE	13.74	Monkey blood	IC 184	VIC	15.07
Milk as such	Cow 184	FAM	18.78	Milk as such	Cow 184	FAM	20.31
Milk as such	IC 184	JOE	14.31	Milk as such	IC 184	VIC	15.26
Human Tissue Formalin	Cow 184	FAM	UD	Human Tissue Formalin	Cow 184	FAM	UD
Human Tissue Formalin	IC 184	JOE	13.88	Human Tissue Formalin	IC 184	VIC	15.1
Human blood	Cow 184	FAM	UD	Human blood	Cow 184	FAM	UD
Human blood	IC 184	JOE	13.64	Human blood	IC 184	VIC	15.04
Human Tissue Normal saline	Cow 184	FAM	UD	Human Tissue Normal saline	Cow 184	FAM	UD
Human Tissue Normal saline	IC 184	JOE	13.82	Human Tissue Normal saline	IC 184	VIC	15.31
Negative Control	Cow 184	FAM	UD	Negative Control	Cow 184	FAM	UD
Negative Control	IC 184	JOE	14.04	Negative Control	IC 184	VIC	15.09
Positive Control	Cow 184	FAM	22.12	Positive Control	Cow 184	FAM	23.28
Positive Control	IC 184	JOE	13.69	Positive Control	IC 184	VIC	15.26

UD: Undetected


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MBPCR210, Hi-PCR® Cow-Buffero Detection Kit

InstaQ 96 Plus (LA1073)				QuantStudio 5			
Sample ID	Target Name	Dye	Ct	Sample ID	Target Name	Dye	Ct
Cow Female Blood	Cow 210	FAM	17.37	Cow Female Blood	Cow 210	FAM	17.53
Cow Female Blood	Buffalo 210	JOE	UD	Cow Female Blood	Buffalo 210	VIC	UD
Cow Female Blood	IC 210	Texas Red	15.07	Cow Female Blood	IC 210	ROX	16.18
Bullock	Cow 210	FAM	18.66	Bullock	Cow 210	FAM	18.82
Bullock	Buffalo 210	JOE	UD	Bullock	Buffalo 210	VIC	UD
Bullock	IC 210	Texas Red	15.52	Bullock	IC 210	ROX	15.68
Buffalo meat	Cow 210	FAM	UD	Buffalo meat	Cow 210	FAM	UD
Buffalo meat	Buffalo 210	JOE	13.87	Buffalo meat	Buffalo 210	VIC	14.97
Buffalo meat	IC 210	Texas Red	14.82	Buffalo meat	IC 210	ROX	16.06
Buffalo Male	Cow 210	FAM	UD	Buffalo Male	Cow 210	FAM	UD
Buffalo Male	Buffalo 210	JOE	19.39	Buffalo Male	Buffalo 210	VIC	20.73
Buffalo Male	IC 210	Texas Red	14.98	Buffalo Male	IC 210	ROX	16.11
Goat meat	Cow 210	FAM	UD	Goat meat	Cow 210	FAM	UD
Goat meat	Buffalo 210	JOE	UD	Goat meat	Buffalo 210	VIC	UD
Goat meat	IC 210	Texas Red	14.94	Goat meat	IC 210	ROX	16.2
Buffalo Female	Cow 210	FAM	UD	Buffalo Female	Cow 210	FAM	UD
Buffalo Female	Buffalo 210	JOE	21.08	Buffalo Female	Buffalo 210	VIC	22.39
Buffalo Female	IC 210	Texas Red	14.95	Buffalo Female	IC 210	ROX	16.04
Chicken meat	Cow 210	FAM	UD	Chicken meat	Cow 210	FAM	UD
Chicken meat	Buffalo 210	JOE	UD	Chicken meat	Buffalo 210	VIC	UD
Chicken meat	IC 210	Texas Red	14.87	Chicken meat	IC 210	ROX	16.1
Milk digested	Cow 210	FAM	19.13	Milk digested	Cow 210	FAM	19.47
Milk digested	Buffalo 210	JOE	UD	Milk digested	Buffalo 210	VIC	UD
Milk digested	IC 210	Texas Red	14.99	Milk digested	IC 210	ROX	16.3
Monkey blood	Cow 210	FAM	UD	Monkey blood	Cow 210	FAM	UD
Monkey blood	Buffalo 210	JOE	UD	Monkey blood	Buffalo 210	VIC	UD
Monkey blood	IC 210	Texas Red	15.09	Monkey blood	IC 210	ROX	16.11
Milk as such	Cow 210	FAM	18.95	Milk as such	Cow 210	FAM	19.01
Milk as such	Buffalo 210	JOE	UD	Milk as such	Buffalo 210	VIC	UD
Milk as such	IC 210	Texas Red	14.93	Milk as such	IC 210	ROX	16.24
Human Tissue Formalin	Cow 210	FAM	UD	Human Tissue Formalin	Cow 210	FAM	UD
Human Tissue Formalin	Buffalo 210	JOE	UD	Human Tissue Formalin	Buffalo 210	VIC	UD
Human Tissue Formalin	IC 210	Texas Red	15.21	Human Tissue Formalin	IC 210	ROX	15.87
Human blood	Cow 210	FAM	UD	Human blood	Cow 210	FAM	UD
Human blood	Buffalo 210	JOE	UD	Human blood	Buffalo 210	VIC	UD
Human blood	IC 210	Texas Red	15.05	Human blood	IC 210	ROX	16.22
Human Tissue Normal saline	Cow 210	FAM	UD	Human Tissue Normal saline	Cow 210	FAM	UD
Human Tissue Normal saline	Buffalo 210	JOE	UD	Human Tissue Normal saline	Buffalo 210	VIC	UD
Human Tissue Normal saline	IC 210	Texas Red	15	Human Tissue Normal saline	IC 210	ROX	16.27
Negative Control	Cow 210	FAM	UD	Negative Control	Cow 210	FAM	UD
Negative Control	Buffalo 210	JOE	UD	Negative Control	Buffalo 210	VIC	UD
Negative Control	IC 210	Texas Red	15.07	Negative Control	IC 210	ROX	16.38
Positive Control	Cow 210	FAM	24.45	Positive Control	Cow 210	FAM	25.13
Positive Control	Buffalo 210	JOE	29.45	Positive Control	Buffalo 210	VIC	31.14
Positive Control	IC 210	Texas Red	15.07	Positive Control	IC 210	ROX	16.38

UD: Undetected


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MBPCR186, Hi-PCR® Cattle Sex Detection Kit

InstaQ 96 Plus (LA1073)

QuantStudio 5

Sample ID	Target Name	Dye	Ct	Sample ID	Target Name	Dye	Ct
Cow Female Blood	X 186	FAM	23.26	Cow Female Blood	X 186	FAM	24.64
Cow Female Blood	Y 186	HEX	UD	Cow Female Blood	Y 186	VIC	UD
Cow Female Blood	IC 186	Texas Red	15.34	Cow Female Blood	IC 186	ROX	15.88
Bullock	X 186	FAM	24.27	Bullock	X 186	FAM	24.36
Bullock	Y 186	HEX	24.01	Bullock	Y 186	VIC	22.86
Bullock	IC 186	Texas Red	15.84	Bullock	IC 186	ROX	15.72
Buffalo meat	X 186	FAM	24.98	Buffalo meat	X 186	FAM	24.92
Buffalo meat	Y 186	HEX	22.72	Buffalo meat	Y 186	VIC	21.29
Buffalo meat	IC 186	Texas Red	15.5	Buffalo meat	IC 186	ROX	15.9
Buffalo Male	X 186	FAM	26.05	Buffalo Male	X 186	FAM	26.11
Buffalo Male	Y 186	HEX	24.28	Buffalo Male	Y 186	VIC	23.24
Buffalo Male	IC 186	Texas Red	15.47	Buffalo Male	IC 186	ROX	16.03
Goat meat	X 186	FAM	UD	Goat meat	X 186	FAM	UD
Goat meat	Y 186	HEX	UD	Goat meat	Y 186	VIC	UD
Goat meat	IC 186	Texas Red	15.05	Goat meat	IC 186	ROX	16.03
Buffalo Female	X 186	FAM	25.7	Buffalo Female	X 186	FAM	26.16
Buffalo Female	Y 186	HEX	UD	Buffalo Female	Y 186	VIC	UD
Buffalo Female	IC 186	Texas Red	15.41	Buffalo Female	IC 186	ROX	15.97
Chicken meat	X 186	FAM	UD	Chicken meat	X 186	FAM	UD
Chicken meat	Y 186	HEX	UD	Chicken meat	Y 186	VIC	UD
Chicken meat	IC 186	Texas Red	15.32	Chicken meat	IC 186	ROX	15.99
Milk digested	X 186	FAM	22.85	Milk digested	X 186	FAM	23.17
Milk digested	Y 186	HEX	UD	Milk digested	Y 186	VIC	UD
Milk digested	IC 186	Texas Red	15.41	Milk digested	IC 186	ROX	15.98
Monkey blood	X 186	FAM	UD	Monkey blood	X 186	FAM	UD
Monkey blood	Y 186	HEX	UD	Monkey blood	Y 186	VIC	UD
Monkey blood	IC 186	Texas Red	15.24	Monkey blood	IC 186	ROX	16
Milk as such	X 186	FAM	23.84	Milk as such	X 186	FAM	24.13
Milk as such	Y 186	HEX	UD	Milk as such	Y 186	VIC	UD
Milk as such	IC 186	Texas Red	15.23	Milk as such	IC 186	ROX	16.06
Human Tissue Formalin	X 186	FAM	UD	Human Tissue Formalin	X 186	FAM	UD
Human Tissue Formalin	Y 186	HEX	UD	Human Tissue Formalin	Y 186	VIC	UD
Human Tissue Formalin	IC 186	Texas Red	15.49	Human Tissue Formalin	IC 186	ROX	15.98
Human blood	X 186	FAM	UD	Human blood	X 186	FAM	UD
Human blood	Y 186	HEX	UD	Human blood	Y 186	VIC	UD
Human blood	IC 186	Texas Red	15.15	Human blood	IC 186	ROX	16.02
Human Tissue Normal saline	X 186	FAM	UD	Human Tissue Normal saline	X 186	FAM	UD
Human Tissue Normal saline	Y 186	HEX	UD	Human Tissue Normal saline	Y 186	HEX	UD
Human Tissue Normal saline	IC 186	Texas Red	15.34	Human Tissue Normal saline	IC 186	Texas Red	15.54
Negative Control	X 186	FAM	UD	Negative Control	X 186	FAM	UD
Negative Control	Y 186	HEX	UD	Negative Control	Y 186	VIC	UD
Negative Control	IC 186	Texas Red	15.2	Negative Control	IC 186	ROX	16.29
Positive Control	X 186	FAM	29.31	Positive Control	X 186	FAM	29.45
Positive Control	Y 186	HEX	25.43	Positive Control	Y 186	VIC	24.5
Positive Control	IC 186	Texas Red	15.41	Positive Control	IC 186	ROX	16.49

UD: Undetected


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HiMedia's Insta Q96® Plus (LA1073)

Sample ID	Sample Type	Cow Uniplex	Buffalo Uniplex	Cow-Buffalo Multiplex	Cattle Sex
		MBPCR184	MBPCR185	MBPCR210	MBPCR186
Cow Female Blood	Blood	Positive	Negative	Cow Positive	Female
Bullock	Blood	Positive	Negative	Cow Positive	Male
Buffalo meat	Tissue	Negative	Positive	Buffalo Positive	Male
Buffalo Male	Blood	Negative	Positive	Buffalo Positive	Male
Goat meat	Tissue	Negative	Negative	Negative	Negative
Buffalo Female	Blood	Negative	Positive	Buffalo Positive	Female
Chicken meat	Tissue	Negative	Negative	Negative	Negative
Milk digested	Milk	Positive	Negative	Cow Positive	Female
Monkey blood	Blood	Negative	Negative	Negative	Negative
Milk as such	Milk	Positive	Negative	Cow Positive	Female
Human Tissue Formalin	Tissue	Negative	Negative	Negative	Negative
Human blood	Blood	Negative	Negative	Negative	Negative
Human Tissue Normal saline	Tissue	Negative	Negative	Negative	Negative

Applied Biosystem's QuantStudio 5

Sample ID	Sample Type	Cow Uniplex	Buffalo Uniplex	Cow-Buffalo Multiplex	Cattle Sex
		MBPCR184	MBPCR185	MBPCR210	MBPCR186
Cow Female Blood	Blood	Positive	Negative	Cow Positive	Female
Bullock	Blood	Positive	Negative	Cow Positive	Male
Buffalo meat	Tissue	Negative	Positive	Buffalo Positive	Male
Buffalo Male	Blood	Negative	Positive	Buffalo Positive	Male
Goat meat	Tissue	Negative	Negative	Negative	Negative
Buffalo Female	Blood	Negative	Positive	Buffalo Positive	Female
Chicken meat	Tissue	Negative	Negative	Negative	Negative
Milk digested	Milk	Positive	Negative	Cow Positive	Female
Monkey blood	Blood	Negative	Negative	Negative	Negative
Milk as such	Milk	Positive	Negative	Cow Positive	Female
Human Tissue Formalin	Tissue	Negative	Negative	Negative	Negative
Human blood	Blood	Negative	Negative	Negative	Negative
Human Tissue Normal saline	Tissue	Negative	Negative	Negative	Negative


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Conclusion

1. All cow positive samples were positive for cow and negative for buffalo by MBPCR184, MBPCR185 and MBPCR210 Real-Time PCR kits.
2. All buffalo positive samples were positive for buffalo and negative for cow by MBPCR184, MBPCR185 and MBPCR210 Real-Time PCR kits.
3. The male and female targets were respectively identified by MBPCR186 Real-Time PCR kit.
4. The human samples and other animal samples apart from cow and buffalo did not show any amplification for the respective targets by MBPCR184, MBPCR185, MBPCR186 and MBPCR210 Real-Time PCR kits.
5. All the 13 samples showed exact results in both the Real-Time PCR machines.
6. Early Ct values were obtained from HiMedia's Insta Q96® Plus (LA1073) as compared to Applied Biosystems™ QuantStudio™ 5.



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