Loopamp Bovine Embryo Sexing Kit
Instruction Manual

《Instruments required》

Detection equipment

・Loopamp End Point turbidimeter LA-100（manufactured by TERAMECS）

Pipettes

・Adjustable micro-pipettes（Adjustable from a few μL～ several hundred μL for sampling manipulation）

（Examples：0.5～10μL, 10～100μL, 100～1000μL）

（If possible, try to use separate pipettes for reaction mix preparation and sample/ positive control handling. ）

・Tip with filter（ RNase/DNase free）

Tips

・Sterilized tubes for master mix preparation（ RNase/DNase free） 1.5mL
・Sterilized tubes for sample pre-treatment（ RNase/DNase free） 0.5mL
・Loopamp reaction tubes, 200μL×８ connected tube

For remaining low temperature

・Equipments used on ice for reaction mix preparation

（Example １：Aluminum rack and crushed ice）
（Example ２：Cold boxes）

・Ice box for sample and positive control handling

Others

・Centrifuge for micro-tubes
・Centrifuge for 8-connected tubes（0.2mL）
・Tube rack（0.2mL, 0.5mL, 1.5mL）
・Vortex mixer（when needed）
・Disposable gloves
・Tips and vinyl bag for sealing the disposed tips（such as uni-pack etc.）

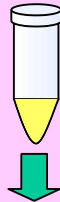
《Bovine Embryo Sexing Kit》

Kit contents（Bovine Embryo sexing kit） for 24 tests（Storage：－20℃ Expiration：in 12 months）		
Extraction Solution	EX	1.5mL×1tube
Reaction Mix. Ⅰ	RM Ⅰ	0.25mL×2tubes
Reaction Mix. Ⅱ	RM Ⅱ	0.25mL×2tubes
Bst DNA polymerase	Bst DNA polymerase	60μL×1tube
Control DNA	Cont. DNA	0.1mL×1tube

Reaction tubes		8×12sets

《Sample Pre-treatment 》

・Thawing the Extraction Solution (EX) at room temperature, mix and spin down.
・Add 6 μ L of biopsy sample solution which containing bovine embryo cells into the sterilized tubes (for pre-treatment use).
・Add Extraction Solution 6 μ L to the tube and mix.
・Keep it at room temperature for above 5 minutes, mix and spin down. The solution is used as sample solution for the reaction.

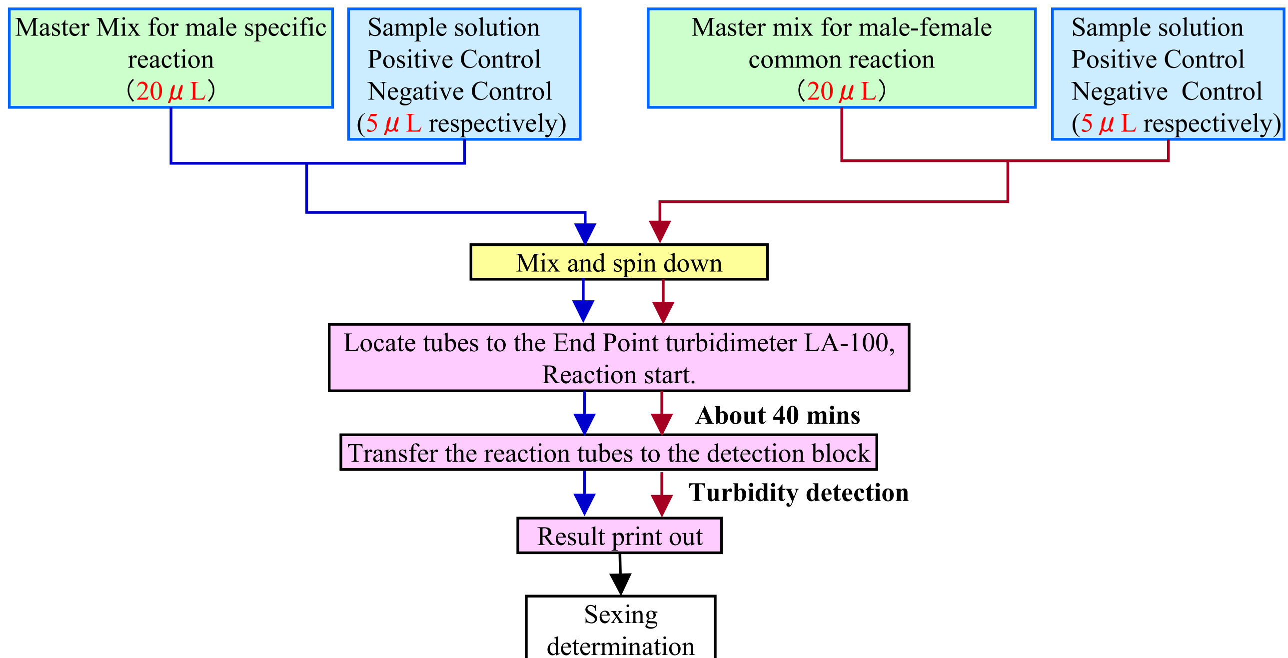


←	Biopsy sample solution	6 μ L
←	Extraction Solution(EX)	6 μ L

Leave at room temperature for at least 5 mins, mix and spin down.

・For biopsy, take 10% from the trophectoderm.
・The volume of biopsy sample should be 6 μ L.
・Avoid opening the cap of Extraction Solution as far as possible

《Operation procedures 》



【Equipment and reagents preparation】

・Before handling the reagents, the End Point turbidimeter LA100 should be set ready for the detection.
・Take out reagents from the refrigerator.
(Thawing RM Ⅰ, RM Ⅱ at room temperature, *Bst* DNA polymerase on ice)

↓

・ Mix RM Ⅰ, RM Ⅱ by vortexing or by tapping, spin down the tube.
・ For *Bst* DNA polymerase, only spin-down is necessary. Vortexing should be avoided.

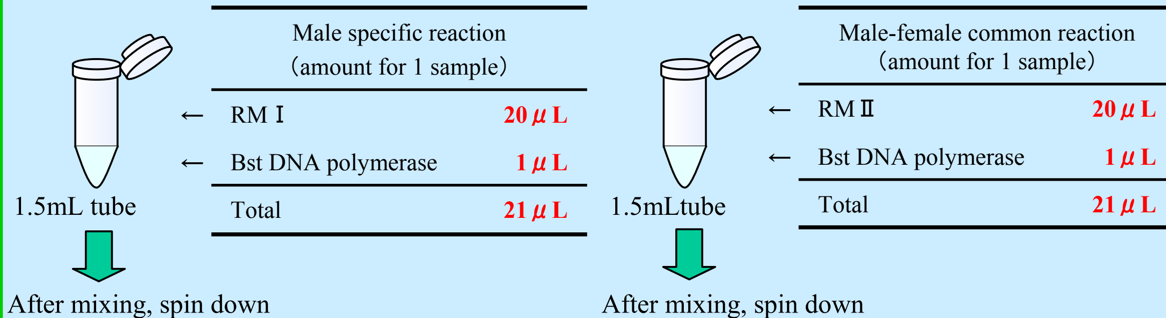
↓

Keep on ice

【Master Mix preparation】

・Add the necessary amount of RMⅠ, *Bst* DNA polymerase into the sterilized tube (for preparing male-specific reaction master mix)
・Add the necessary amount of RMⅡ, Bst DNA polymerase into the sterilized tube (for preparing male-female common reaction master mix)
・Mix by tapping,or by repeatedly reversing mixing,or by vortexing(1second X 3 times) *; spin down the tube.

* Too much fierce mixing by vortex mixer might inactivate the polymerase.

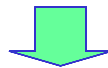


Male specific reaction (amount for 1 sample)	
RMⅠ	**20μL**
Bst DNA polymerase	**1μL**
Total	**21μL**

Male-female common reaction (amount for 1 sample)	
RMⅡ	**20μL**
Bst DNA polymerase	**1μL**
Total	**21μL**

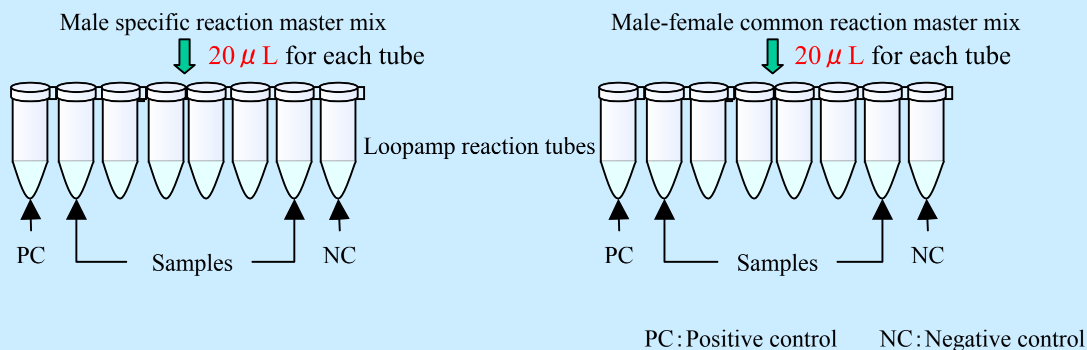
Example: in the case of handling 6 samples
Prepare the amount for 8 tubes including PC (positive control), NC (negative control), and samples

RM	20μL×8=160μL
Bst DNA polymerase	1μL×8= 8μL
Total	168μL



【Master mix dispensing】（Reference: Cautions for Pipette manipulation（example 1））

・Check to confirm there is no scratch or crack on the Loopamp reaction tubes.
・Dispense **20μL** of the prepared master mix into each reaction tube and lightly close all caps.
・The reaction tubes containing the master mix should be kept on ice inside the clean bench.



PC: Positive control NC: Negative control

《Cautions for handling tubes》

・Please only use the reaction tubes exclusive for the kit. The usage of other reaction tubes might cause misjudgment.
・Reaction tubes are vulnerable to scratches, please handle with care. Damaged tubes can contaminate the tubidimeter, and the equipment might be disqualified for future use.
・The sterilized tubes (for master mix preparation) and the reaction tubes should not be exposed to UV light for sterilization. UV light can deteriorate the tube material and cause damages or color changes. These changes might cause non-specific amplification and lead to false results.

【The preparation of controls and sample solution】

【Positive control】

・Thaw the Control DNA（Cont.DNA）at room temperature, mix and spin down, and then keep it on ice.

【Negative control】

・Mix Extraction Solution and blastomere washing solution (or biopsy buffer solution) at the ratio １:１. Mix well, spin down and keep on ice.

【Sample solution】

・Keep the sample solution on ice.

＊Positive control can cause contamination, please take extra care.
If the spin-down is not sufficient, the solution staying on the cap interior can disperse and cause contamination.

【Adding negative control】（Reference: Cautions for pipetting manipulation（Example 2））

・Open the cap of the tube containing negative control.

↓

・Perform the pipetting manipulation once within the tube and then aspirate **5μL**.

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・Move the pipette to the reaction tube. While slowly rotating the pipette within the tube, perform pipetting manipulation for 5〜10 times.

＊Please prevent the solution from being stuck to the inner tube wall.

↓

・Finally, push the pipette's button to its 1st stop position to dispense the inside solution, and then remove the pipette.

＊Dispose the tip.

＊Perform the procedures to male specific reaction and male-female common reaction respectively.

【Adding sample solution】

・Open the cap of the tube containing sample solution.

↓

・Perform the pipetting manipulation once within the tube and then aspirate **5μL**.

↓

・Move the pipette to the reaction tube. While slowly rotating the pipette within the tube, perform pipetting manipulation for 5〜10 times .

＊ Please prevent the solution from being stuck to the inner tube wall.

↓

・Finally, push the pipette's button to its 1st stop position to dispense the inside solution, and then remove the pipette.

＊Dispose the tip.

＊Perform the procedures to male specific reaction and male-female common reaction respectively

【Adding positive control】

・Lastly, open the cap of the tube containing positive control.

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・Use the pipette which is exclusive for the positive control, (the procedures are the same as adding negative control and sample solution), to add **5μL** Control DNA into the reaction tube.

＊Dispose the tip.

＊Conduct the procedures to male specific reaction and male-female common reaction respectively.

【Confirmations before amplification reaction】

・Confirm the solution volume inside the reaction tubes.
（Correct dispensing will assure that all dispensed solution is in the same volume, and the yellow color can be seen.）
・Spin down and confirm no bubble inside the tubes.
・Confirm the reaction block in the turbidimeter has reached the temperature of 63.0℃.

【Amplification reaction and detection】

・Locate the reaction tubes to the end-point turbidimeter LA-100, press【START/ENTER】button.
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・Reaction proceeds at 63.0℃ for 35 minutes. And after the reaction, inactivate the enzyme at 80℃ for 2 minutes.
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・While the message《MEASURE》is flashing, remove the reaction tubes from reaction block to the detection block. Press【START/ENTER】button.
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・Detection

【Judging standard】

・Confirm that positive control reaction shows a「＋」and negative control shows a「－」sign.
・Male/ female determination:

	RMⅠ（male specific reaction）	RMⅡ（male-female common reaction）	Judgment
results	＋	＋	male
	－	＋	female
	＋	－	retest
	－	－	

《Cautions for locating reaction tubes》

Confirm that the tubes are not scratched, foggy, and no foreign matter or bubble inside.
Locate the reaction tubes firmly to the reaction block and the detection block.
Gently close the hot bonnet and the cover of the detection block .

《Cautions for handling amplification products》

・After the detection, please wear gloves to remove the reaction tubes.
・The reaction tubes are vulnerable to damages, please handle with care.
・After the reaction, the cap of the tubes should not be open, especially while removing the reaction tubes from the turbidimeter.
・The disperse of amplification products will not only contaminate the turbidimeter and cause false judgment, but also disqualify the equipment for future use.
・After the reaction, keep the caps of the tubes closed and carry out incineration or dispose the tubes by sealing them in a vinyl bag.
・In order to avoid dispersing the amplification products, do not carry out high pressure sterilization on disposing the tubes.

(Reference) Cautions for pipetting manipulation

【 Recommended procedures for pipetting manipulation on LAMP 】

On carrying out LAMP reactions, pipetting manipulation is required for dispensing of master mix, adding samples/ controls, aspirating and discharging minute amount of solution.

Performing the usual pipetting manipulation operation (pushing the button up to the 2nd-stop position on discharging), it can cause bubbles, which are uneasy to remove.

Therefore, it is recommended to push the button only up to the 1st stop position on dispensing master mix to reaction tubes.

Example 1 : Dispensing 20 μL master mix to each Loopamp reaction tube

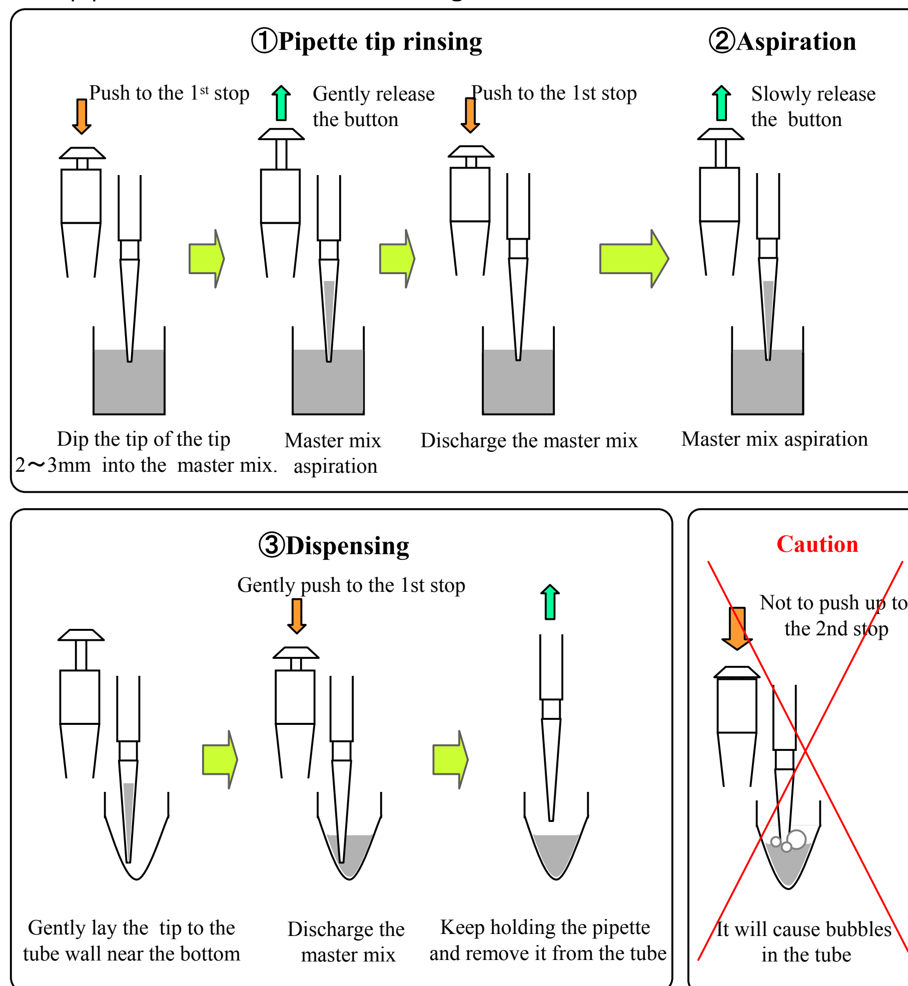
① Firstly, rinse the pipette tip once with the master mix: Push the pipette's button to the 1st stop position, dip the tip 2～3mm into the master mix solution, gently perform aspiration, and then push the button to the 1st stop position.

② Keep tip end at the same position and gently aspirate 20 μL from the master mix, and then hold the pipette for a few seconds.

③ Transfer the pipette carrying master mix to the reaction tube. Try to slightly place the tip end on the inner tube wall near the bottom, and gently push the button to the 1st stop position. Keep holding the pipette and slowly remove it from the tube.

Although this allows a minute amount of master mix to be left in the tip, this should not be a problem for the detection. Keep holding the pipette and, for the next dispensing, dip the tip into the master mix to aspirate 20 μL, remove the pipette and repeat step ③ to dispense it into the reaction tube. Repeat the procedures to dispense the master mix into each reaction tube.

＊The attempt to discharge all the master mix by pushing the pipette button to the end stop (push up to the 2nd stop position) will result in the shortage of master mix or cause bubbles in the tubes.



Example 2：Adding 5 μL sample/controls into the reaction tube

① Firstly, rinse the tip once with the sample solution: Push the pipette's button to the 1st stop position, dip the tip 2～3mm into the sample solution, gently perform aspirating and then push the button to the 1st stop position.

② Let the tip stay at the same position and slowly aspirate 5 μL from the sample solution.

③ Transfer the pipette carrying sample solution to the reaction tube. Dip the tip 2～3mm into the master mix in the reaction tube, gently push to the 1st stop position to discharge the sample. And then release the button, while lightly rotating the tip end in the solution, rinse the tip for 5～10 times according to the procedures in ① (The same times of rinsing operation should be done to each tube). Finally, gently push to the 1st stop position, keep holding the pipette and then remove the tip from the tube.

＊Pushing up to the 2nd stop might cause bubbles in the tube.