Comparison Test of Performance of Proteinase K Stored Cold and Stored Room Temp.  
Tissue DNA Extraction Test

Objective
To compare the performance of cold-stored Proteinase K (-20°C) and room temperature-stored Proteinase K (25-28°C) used in tissue DNA extraction test.

Passing Criteria
The reading of nucleic acid is detected and correspondence to absorbance value limit for A260 wavelength. Corresponding absorbance value limits for A260 is within the range of 0.01 to 1.6 Abs and for A260/280 is greater than 1.7.  
The amplification of extracted DNA using conventional PCR showed positive results with 350bp band.  
The amplification of extracted DNA using real-time PCR showed positive results with the difference of Ct value between two Proteinase Ks less than 3.

Samples
- Beef tissue sample (tripe meat)
- Chicken tissue sample (chicken breast)
- Pork tissue sample (lean meat)

Protocol

A. DNA Extraction from Cultured Animal Cells

B. DNA Extraction from Animal Tissue

Addition of ethanol
Add 200ul absolute ethanol and mix immediately.

Loading to column
Transfer sample to column

Centrifuge
5,000 x g 1 min

Column washing
Add 0.5ml Wash Buffer

Centrifuge
5,000 x g 1 min

Centrifuge
10,000 x g 1 min

Column drying
Transfer column to a new microcentrifuge tube.
Add 200ul preheated Elution Buffer or water. Stand for 2 min.

Elution

Centrifuge
5,000 x g 1 min

Store DNA at 4°C or 30°C

Transfer sample to column

Optional: Removal of RNA
Add 20ul RNase A. Incubate 37°C, 10 min.

Homogenization
Add 2 volumes Buffer TB and mix thoroughly. Incubate 65°C, 10 min.

Optional: Cell lysis
Add 2ml of Proteinase K. Mix by pulsed-vortexing.

Add 20ul of Lysis Enhancer. Add 200ul of Buffer TB. Mix by pulsed-vortexing.

Cell lysis

Add 20ul of Proteinase K.

Add 200ul of PDS and resuspend completely.

Centrifugation and resuspension
Pellet cells at 600 x g for 5 min. Add 200ul of PDS and resuspend completely.

Tissue preparation
Cut tissue into small pieces or grind into the powder in liquid nitrogen.

Discard through.
Add 650ul Wash Buffer.

Discard through. Repeat again washing step.
Results

Beef Tissue Sample

Legend:
C1&C2: Extracted DNA with more than 50ng/µl; extraction using cold stored Proteinase K
R1&R2: Extracted DNA with more than 50ng/µl; extraction using room temperature stored Proteinase K
M: VC 1kb DNA ladder

Figure 1: 2µl of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

Chicken Tissue Sample

Legend:
C1&C2: Extracted DNA with more than 30ng/µl; extraction using cold stored Proteinase K
R1&R2: Extracted DNA with more than 30ng/µl; extraction using room temperature stored Proteinase K
M: VC 1kb DNA ladder

Figure 2: 2µl of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

Pork Tissue Sample

Legend:
C1&C2: Extracted DNA with more than 30ng/µl; extraction using cold stored Proteinase K
R1&R2: Extracted DNA with more than 30ng/µl; extraction using room temperature stored Proteinase K
M: VC 1kb DNA ladder

Figure 3: 2µl of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.
**Downstream Application**
Conventional PCR and real-time PCR were carried out using the extracted DNA. Both tests were performed using tissue universal primer.

**Beef Tissue Sample**

![Image]

Legend:
M: 100bp DNA ladder
C1&C2: Amplification product using extracted DNA which used cold-stored Proteinase K in extraction
R1&R2: Amplification product using extracted DNA which used room temperature-stored Proteinase K in extraction

**Figure 4**: 2µl of extracted DNA was used for amplification. 5µl of PCR product was loaded into 1% TBE gel. The expected band size is 350bp.

![Graph](image)

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample</th>
<th>Sample Name</th>
<th>Gene</th>
<th>Ct</th>
<th>Mean Ct</th>
<th>Conc. St.</th>
<th>Mean Co</th>
<th>Std. Dev.</th>
<th>Std. Dev.</th>
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**Figure 5**: 2µl of extracted DNA was used for real-time amplification. According to the graph and table on top, the difference in Ct value between two different Proteinase Ks is 0.220.

| Mean Ct value for RT Beef | 24.495 |
| Mean Ct value for Cool Beef | 24.715 |
| Difference Ct value between RT and Cool | 0.220 |
Chicken Tissue Sample

Legend:
M: 100bp plus DNA ladder
C1&C2: Amplification product using extracted DNA which used cold-stored Proteinase K in extraction
R1&R2: Amplification product using extracted DNA which used room temperature-stored Proteinase K in extraction
-ve: Amplification product with no extracted DNA

Figure 6: 2µl of extracted DNA was used for amplification. 5µl of PCR product was loaded into 1% TBE gel. The expected band size is 350bp.

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample</th>
<th>Sample</th>
<th>Gene</th>
<th>Ct</th>
<th>Mean Ct</th>
<th>Conc. St.</th>
<th>Mean CoT</th>
<th>Std. Dev.</th>
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Mean Ct value for RT Chicken: 22.300
Mean Ct value for Cool Chicken: 22.385
Difference Ct value between RT and Cool: 0.085

Figure 7: 2µl of extracted DNA was used for real-time amplification. According to the graph and table showed on top, the difference in Ct value between two different Proteinase Ks is 0.085
Pork Tissue Sample

Legend:
M: 100bp plus DNA ladder
C1&C2: Amplification product using extracted DNA which used cold-stored Proteinase K in extraction
R1&R2: Amplification product using extracted DNA which used room temperature-stored Proteinase K in extraction

**Figure 8:** 2µl of extracted DNA was used for amplification. 5µl of PCR product was loaded into 1% TBE gel. The expected band size is 350bp.

<table>
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<th>Well</th>
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**Figure 9:** 2µl of extracted DNA was used for real-time amplification. According to the graph and table showed on top, the difference Ct value between two different Proteinase Ks is 0.84.
Conclusion

3 different tissue samples were extracted using GF-1 Tissue DNA Extraction kit. From the gel photos, there was no significant difference showed in the performance of Proteinase K that was stored in either cold or room temperature condition as the results of amplifications of extracted DNA using conventional PCR showed no significant different for bands; and using real-time PCR showed that all differences between the two Proteinase Ks are within 1Ct value. The sensitivity of the conventional and real-time assay was not affected by the use of room temperature-stored Proteinase K.

Prepared by,

Vivantis Technical Team

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