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|  | **OPERASI PERKHIDMATAN SOKONGAN**  **FAKULTI PERUBATAN VETERINAR**  **(UNIT PERKHIDMATAN MAKMAL VETERINAR)**  **Kod Dokumen: OPR/FPV/BR113/BHLW** |
| **VIROLOGY 3/HiCOE IBS LABORATORY WORKSHEET** |

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| --- | --- | --- |
| **Lab. Ref. No. :** | **Patient ID:** | **Case No.:** |
| **Date:** | **Test Required:** | **No. of Specimens:** |
| **Time:** | **Type of Specimens:** | |

**Reaction Mixture for 1 Step RT-PCR**

**i)Avian Influenza Virus (AIV)**

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| **Reaction Mixture** | **Final Concerntration** | **Volume for 1X Reaction (µl)** | **Volume for \_\_\_\_\_\_Reaction (µl)** |
| a)AMV/*Tfl* 5X reaction buffer | 1X | 5.0 |  |
| b)MgSO4 (25mM) | 3mM | 3.0 |  |
| c)dNTP Mix (10mM each) | 0.2mM | 0.5 |  |
| d)Forward primer (10µM) – NP1200F | 0.2µM | 0.5 |  |
| e)Reverse primer (10µM) – NP1529R | 0.2µM | 0.5 |  |
| f)RNasin® Ribonuclease Inhibitor (40µ/µl) | 0.8µ/µl | 0.5 |  |
| g)AMV Reverse Transcriptase (5µ/µl) | 0.1µ/µl | 0.5 |  |
| h)*Tfl* DNA Polymerase (5µ/µl) | 0.1µ/µl | 0.5 |  |
| i)RNA template | 10ng/µl-1µg/µ/ | 2.0 |  |
| j)Nuclease Free water (to a final volume of 25µl) | - | Y |  |
| **TOTAL** |  | **25.0** |  |

**ii)Infectious Bronchitis Virus (IBV)**

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| --- | --- | --- | --- |
| **Reaction Mixture** | **Final Concerntration** | **Volume for 1X Reaction (µl)** | **Volume for \_\_\_\_\_\_Reaction (µl)** |
| a)AMV/*Tfl* 5X reaction buffer | 1X | 5.0 |  |
| b)MgSO4 (25mM) | 2mM | 2.0 |  |
| c)dNTP Mix (10mM each) | 0.2mM | 0.5 |  |
| d)Forward primer (20µM) – N104F | 0.4µM | 0.5 |  |
| e)Reverse primer (20µM) – N101R | 0.4µM | 0.5 |  |
| f)RNasin® Ribonuclease Inhibitor (40µ/µl) | 0.8µ/µl | 0.5 |  |
| g)AMV Reverse Transcriptase (5µ/µl) | 0.1µ/µl | 0.5 |  |
| h)*Tfl* DNA Polymerase (5µ/µl) | 0.1µ/µl | 0.5 |  |
| i)RNA template | 10ng/µl-1µg/µ/ | 1.0 |  |
| j)Nuclease Free water (to a final volume of 25µl) | - | Y |  |
| **TOTAL** |  | **25.0** |  |

**iii)Infectious Bursa Disease Virus (IBDV)**

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| --- | --- | --- | --- |
| **Reaction Mixture** | **Final Concerntration** | **Volume for 1X Reaction (µl)** | **Volume for \_\_\_\_\_\_Reaction (µl)** |
| a)AMV/*Tfl* 5X reaction buffer | 1X | 5.0 |  |
| b)MgSO4 (25mM) | 3mM | 3.0 |  |
| c)dNTP Mix (10mM each) | 0.2mM | 0.5 |  |
| d)Forward primer (25µM) – IFVVC | 0.5µM | 0.5 |  |
| \*e)Reverse primer (25µM) – RCLA(D78)  - IVIR (94) | 0.5µM  0.5 µM | 0.5  0.5 |  |
| f)RNasin® Ribonuclease Inhibitor (40µ/µl) | 0.8µ/µl | 0.5 |  |
| g)AMV Reverse Transcriptase (5µ/µl) | 0.1µ/µl | 0.5 |  |
| h)*Tfl* DNA Polymerase (5µ/µl) | 0.1µ/µl | 0.5 |  |
| i)RNA template | 10ng/µl-1µg/µ/ | 2.0 |  |
| j)Nuclease Free water (to a final volume of 25µl) | - | Y |  |
| **TOTAL** |  | **25.0** | **/ 2 =** |

**\*Divide by two the total volume of Reaction Mixture into another 1.5ml microcentrifuge tube and add reverse primer (depend on number of sample) into each tube**

**iv)Newcastle Disease Virus (NDV)**

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| **Reaction Mixture** | **Final Concerntration** | **Volume for 1X Reaction (µl)** | **Volume for \_\_\_\_\_\_Reaction (µl)** |
| a)5X Flexi RT buffer | 1X | 5.0 |  |
| b) MgCl2(25mM) | 2mM | 2.0 |  |
| c) dNTP Mix (10mM each) | 0.2mM | 0.5 |  |
| d) Forward primer (20µM) – NDVIF2 | 0.2µM | 0.5 |  |
| \*e) Reverse primer (20µM) – NPV2N  - NPL2N | 0.2µM  0.2µM | 0.5  0.5 |  |
| f) RNasin® Ribonuclease Inhibitor (40µ/µl) | 0.8µ/µl | 0.5 |  |
| g) AMV Reverse Transcriptase (5µ/µl) | 0.1µ/µl | 0.5 |  |
| h)*Taq* DNA Polymerase (5µ/µl) | 0.1µ/µl | 0.5 |  |
| i)RNA template | 10ng/µl-1µg/µ/ | 2.0 |  |
| j) Nuclease Free water (to a final volume of 25µl) | - | Y |  |
| **TOTAL** |  | **25.0** |  |

**\*Divide by two the total volume of Reaction Mixture into another 1.5ml microcentrifuge tube and add reverse primer (depend on number of sample) into each tube**

**Reaction Mixture of PCR**

**i)*Mycoplasma Gallisepticum* Disease**

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| --- | --- | --- | --- |
| **Reaction Mixture** | **Final Concerntration** | **Volume for 1X Reaction (µl)** | **Volume for \_\_\_\_\_\_Reaction (µl)** |
| a)10X PCR Buffer | 1X | 2.5 |  |
| b)MgCl2 (25mM) | 2mM | 1.75 |  |
| c)dNTP Mix (10mM) | 0.2mM | 1.0 |  |
| d)Forward primer (10uM) – MG14F | 0.2µM | 0.5 |  |
| e)Reverse primer (10uM) – MG13R | 0.2µM | 0.5 |  |
| f)Taq polymerase (5u/ul) | 0.1µ/µl | 0.2 |  |
| g)DNA template | 1µg | 2.0 |  |
| h)Nuclease free water (to a final volume of 25µl) | - | Y |  |
| **TOTAL** |  | **25.0** |  |

**ii)DNA Sexing**

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| --- | --- | --- | --- |
| **Reaction Mixture** | **Final Concerntration** | **Volume for 1X Reaction (µl)** | **Volume for \_\_\_\_\_\_Reaction (µl)** |
| a)10X PCR Buffer | 1X | 2.5 |  |
| b)MgCl2 (25mM) | 2mM | 1.75 |  |
| c)dNTP Mix (10mM) | 0.2mM | 1.0 |  |
| d)Forward primer (10uM) – P8 | 0.2µM | 0.5 |  |
| e)Reverse primer (10uM) – P2 | 0.2µM | 0.5 |  |
| f)Taq polymerase (5u/ul) | 0.1µ/µl | 0.2 |  |
| g)DNA template | 1µg | 2.0 |  |
| h)Nuclease free water (to a final volume of 25µl) | - | Y |  |
| **TOTAL** |  | **25.0** |  |

**RESULT:**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Name of Technical Staff: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Sign:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Approved by: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**(sign&chop)**