

## ABSTRAK

### **EKSPRESI PROTEIN *FUSION* REKOMBINAN VIRUS *NEWCASTLE DISEASE* DARI PLASMID *ESCHERICHIA COLI* BL21 (BE3) KLON C-1B MENGGUNAKAN *Accurapid™ Cell-Free Protein Expression Kit***

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*Newcastle Disease* (ND) adalah penyakit yang menyerang unggas yang disebabkan oleh virus *Newcastle Disease*. Virus ND merupakan virus dari famili *Paramyxoviridae* yang memiliki amplop dengan genom *ss-RNA sense* negatif yang tidak bersegmen. Virus ND memiliki enam gen penyandi protein yaitu *nucleoprotein* (N), *phosphoprotein* (P), *matrix protein* (M), protein *fusion* (F), *hemagglutinin-neuraminidase* (HN), dan protein *polymerase* (L). *Fusion* protein merupakan protein dari virus ND yang memiliki peranan penting dalam proses infeksi virus ND ke hospes. Protein *Fusion* rekombinan virus ND dapat menginduksi respon imun sel hospes sehingga berpotensi menjadi vaksin virus ND. Plasmid pBT7-N-His-F merupakan plasmid dari bakteri *Escherichia coli* BL21 (BE3) yang telah disisipi gen penyandi protein F virus ND. Protein F virus ND yang disisipkan merupakan hasil isolat lokal Galur, Kulon Progo (0663/04/2013). Penelitian ini bertujuan mengekspresikan plasmid pBT7-N-His-F menjadi protein F rekombinan virus ND. Plasmid yang digunakan sebagai sampel adalah plasmid pBT7-N-His-F klon C-1b. Elektroforesis DNA sampel plasmid menggunakan gel agarosa 1% selama 45 menit dengan tegangan 80 V menunjukkan pita DNA ukuran 4.643 bp yang menunjukkan terdapat plasmid pBT7-N-His-F. Sampel dipotong dengan enzim *EcoR1* pada suhu 37°C selama 4 jam untuk memisahkan vektor pBT7-N-His dengan sisipan protein F virus ND. Visualisasi hasil pemotongan dilakukan elektroforesis DNA menggunakan gel agarosa 1% selama 1 jam dengan tegangan 80 V. Hasil elektroforesis DNA didapatkan pita DNA ukuran 4001 bp yang merupakan vektor pBT7-N-His dan 642 bp yang merupakan sisipan gen penyandi protein F virus ND. Ekspresi protein F rekombinan dari sampel menggunakan *AccuRapid™ Cell-free Expression Kit* dilakukan selama 3 jam dalam suhu 30°C. Visualisasi hasil ekspresi protein F rekombinan sampel dilakukan *Sodium Dodecyl Sulfat - Polyacrylamide Gel Electrophoresis* (SDS-PAGE) dengan gel *polyacrylamide* 12% selama 2 jam dengan tegangan 100 V. Hasil SDS-PAGE merupakan protein dengan berat 25,6 kDa yang menunjukkan protein F rekombinan virus ND berhasil terekspresikan. Hasil SDS-PAGE dilakukan *Western blot* menggunakan membran *Polyvinyl Difluoride* (PVDF) selama 1 jam dengan tegangan 500 mA. Hasil visualisasi dengan penambahan substrat didapatkan protein dengan berat molekul 25,6 kDa yang menunjukkan protein F rekombinan virus ND berhasil terekspresi.

**Kata kunci :** *Newcastle disease*, pBT7-N-His-F, *EcoR1*, *Cell-free protein expression*

## ABSTRACT

### **EXPRESSION OF NEWCASTLE DISEASE VIRUS FUSION PROTEIN RECOMBINANT FROM ESCHERICHIA COLI BL21 (BE3) CLONE C-1B PLASMID USING *Accurapid™ Cell-Free Protein Expression Kit***

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*Newcastle Disease* (ND) is an infectious disease that infected poultry caused by *Newcastle Disease Virus* (NDV). The NDV is a virus from family *Paramyxoviridae* which enveloped, unsegmented ss-RNA negative sense. The NDV has six protein coding gene there are Nucleoprotein (N), Phosphoprotein (P), Matrix protein (M), Fusion protein (F), Hemagglutinin-Neuraminidase (HN), and Polymerase protein (L). Fusion protein is a protein from NDV that has an important role in the NDV infection process to the host. Fusion protein recombinant of NDV can induce the immune response of the host cell so that it has the potential to become an NDV vaccine. Plasmid pBT7-N-His-F is a plasmid from the *Escherichia coli* BL21 (BE3) that has been inserted with NDV F protein coding gene. The Insert is a Fusion NDV protein from local isolate of NDV Galur, Kulon Progo (0663/04/2013). The purpose of this study is to expressing plamid pBT7-N-His-F NDV to protein F recombinant. Plasmids used as samples are plasmid pBT7-N-His-F clones C-1b. DNA electrophoresis of plasmid samples using 1% agarose gel for 45 minutes with a voltage of 80 V produced a 4,642 DNA band showed that there was a plasmid pBT7-N-His-F in sample. Samples were cut with *EcoR1* enzyme at 37°C for 4 hour to separate the pBT7-N-His vector with F protein NDV insert. Cutting results were visualized by DNA electrophoresis using 1% agarose gel for 1 hour with 80 V. The results of DNA electrophoresis obtained 4001 bp DNA band which was a pBT7-N-His vector and 642 bp which was the insert of the F protein encoding gene. The expression of recombinant F protein from samples using *AccuRapid™* Cell-free Expression Kit for 3 hours at 30°C. The visualization of the recombinant F protein expression samples was using Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) with 12% polyacrylamide gel for 2 hours with 100 V. The results of SDS-PAGE were 25.6 kDa weight proteins that showed recombinant F protein NDV successfully expressed. SDS-PAGE results used as a material of Western blot using Polyvinyl Difluoride (PVDF) membranes for 1 hour with a voltage of 500 mA. The results visualized with the addition of substrate showed 25.6 kDa protein which showed that NDV recombinant protein was successfully expressed.

**Keywords:** Newcastle disease, pBT7-N-His-F, *EcoR1*, Cell-free protein expression