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## EXPRESSION OF RECOMBINANT ANTIBODY FRAGMENT, ANTI BNP-SCFV ON THE PERIPLASM OF *Escherichia coli* FOR THE DETECTION OF HEART FAILURE

### EKSPRESI FRAGMENT ANTIBODI REKOMBINAN, ANTI BNP-SCFV DALAM PERIPLASMA INANG *Escherichia coli* UNTUK DETEKSI GAGAL JANTUNG

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#### ABSTRAK

*Basic natriuretic peptide* (BNP) merupakan polipeptida yang terdiri dari 32 asam amino yang disekresikan oleh bilik jantung untuk merespon peregangan yang berlebihan pada sel otot jantung. BNP berpotensi untuk digunakan sebagai marker untuk meramalkan pasien yang mengalami gagal jantung. Keberadaan BNP dalam darah dapat dideteksi menggunakan antibodi terhadap BNP yaitu Anti BNP-*single chain variable fragment* (anti BNP-SCFV). Fragmen antibodi ini merupakan gabungan polipeptida antara daerah yang bervariasi pada rantai *heavy* (V<sub>H</sub>) dan rantai *light* (V<sub>L</sub>) dari immunoglobulin. Anti BNP-SCFV akan berinteraksi dengan BNP melalui pengenalan antigen-antibodi. Konsentrasi BNP dalam darah pasien dapat dideteksi melalui interaksi BNP dengan Anti BNP-SCFV menggunakan metoda immunosensor. Produksi anti BNP-SCFV rekombinan dalam inang *Escherichia coli* dilaporkan pada penelitian ini. Anti BNP-SCFV di ekspresikan dalam bentuk fusi dengan peptida sinyal OmpC yang berfungsi untuk mengarahkan ke membran periplasma. Ekspresi dilakukan dibawah promotor RhaBAD dengan menggunakan L-rhamnosa sebagai penginduksi. Karakterisasi dengan SDS-PAGE memperlihatkan pita konsisten pada 28 kDa, yang diasumsikan Anti BNP-SCFV. Kondisi optimum ekspresi adalah empat jam setelah induksi dengan konsentrasi penginduksi 4 mM. Anti BNP-SCFV disekresikan ke luar sel, yang dikarakterisasi dengan ditemukannya fraksi protein pada membran periplasma dan medium kultur.

**Kata kunci:** Anti BNP-SCFV, periplasma, *Escherichia coli*, BNP

#### ABSTRACT

*Basic natriuretic peptide* (BNP) is a polypeptide hormone consist of 32 amino acids that secreted by the heart ventricle to respond the excessive stretching of heart muscle cells. BNP can be used as prognostic marker for patients with heart failure. The presence of BNP in blood can be detected by BNP antibody, which is anti BNP-single chain variable fragment (Anti BNP-SCFV). The antibody is a combination of polypeptides between varying region on the heavy chain (V<sub>H</sub>) and the light chain (V<sub>L</sub>) of immunoglobulin. Anti BNP-SCFV will bind to BNP through the antigen-antibody interaction. Concentration of BNP in a patient's blood can be detected through the interaction of BNP with Anti BNP-SCFV using immunosensor method. Production of recombinant Anti BNP-SCFV in *Escherichia coli* as host is reported in the present study. Anti BNP-SCFV was expressed in fusion form with OmpC signal peptide that direct the protein to a periplasmic space. Expression was performed under RhaBad promoter as control using L-rhamnose as inducer. SDS-PAGE characterization showed consistent band at 28 kDa, which was assumed as Anti BNP-SCFV. The optimum expression was found at four hours after induction with 4 mM inducer. Anti BNP-SCFV was secreted from the cell as characterized by the presence of the protein on periplasmic membrane and extracellular fraction.

**Key words:** Anti BNP-SCFV, periplasmic, *Escherichia coli*, BNP.

#### INTRODUCTION

Heart failure is a condition in which the amount of blood pumped by heart is unable to meet the requirement of normal body's metabolism. This disease is characterized by hemodynamic disturbances where the amount of blood in the heart reduced and increased of

ventricular filling pressure is observed (Francis, Gassler & Sonnenblick, 2005).

BNP (B-type natriuretic peptide) is a neuro-hormone secreted primarily in the heart ventricle to respond the increased volume and pressure of the heart. The presence of BNP is useful for diagnosis of heart failure