

# Molecular cloning and expression of cDNAs encoding two novel orthologs of MIF (macrophage migration inhibitory factor) from *D. repens* using prokaryotic and eucaryotic expression systems

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*Dirofilaria repens* is a parasite of domestic and wild canids, other carnivorous and occasionally human (it has a zoonotic potential). Dirofilariasis is endemic in many countries of the Old World. Adult filarial nematodes locate in the subcutaneous and intramuscular tissue. It is a vector-borne parasitosis – an intermediate host is a mosquito from the genus *Aedes*, *Culex*, and *Anopheles*.

Orthologues of human MIF (macrophage migration inhibitory factor) have been found in numerous parasite species, in particular, filarial nematodes, including *Brugia*, *Wuchereria* and *Onchocerca* genus. Mammalian MIF is a pro-inflammatory cytokine with pleiotropic functions and the key regulator of inflammatory immune response. The role of MIF orthologues from these organisms is not definitely understood although

several reports indicated that they have important function in immune evasion strategies, immunomodulation in the host, e.g., differential activation of macrophages.

In the present study, we have cloned and sequenced a full length cDNAs of two novel MIFs from *D. repens* using RACE-PCR. cDNAs encoding two antigens were cloned into pET-28a and pPICZ A plasmid. The recombinant proteins were produced in *Escherichia coli* (BL-21) and *Pichia pastoris* (X33) cells and purified using affinity nickel chromatography.

Due the role of modulating immune system by MIFs, the recombinant proteins may be used, in the future, as drug targets or vaccine antigen against dirofilariasis.