

## 小鼠FANCL 抗体制备及组织表达谱分析

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**摘要** FANCL 是一个可与泛素结合蛋白, 它作为泛素E3连接酶催化FANCD2的单一泛素化。在修复DNA损伤维持染色体稳定的P4途径中起着关键作用。近期发现FANCL与小鼠原始生殖细胞增殖密切相关。成年睾丸中FANCL与几个生殖细胞特异性蛋白形成一个蛋白复合体, 可能参与影响精子的生成。采用RT-PCR方法从小鼠总RNA中扩增克隆FANCL全长cDNA片段, 构建表达质粒, 在大肠杆菌中表达了His-FANCL蛋白, 用该蛋白作为抗原免疫新西兰白兔制备了抗FANCL多抗血清。采用磁离子金属螯合柱纯化His-FANCL蛋白后, 通过与活性基团-NHS交联制备了FANCL抗原柱, 亲和纯化了FANCL多抗。为了验证抗体活性和特异性, 在HEK 293T细胞中瞬时表达了His-FANCL融合蛋白, 分别用His单抗和纯化多抗进行Western印迹分析, 结果表明获得了特异性抗FANCL抗体。为了观察FANCL在组织中的表达谱, 我们制备了多种小鼠组织匀浆蛋白, 使用纯化的FANCL多抗进行Western印迹分析, 在脑、心、肺、肝、脾、肾、睾丸、卵巢、子宫和肌肉组织中都检测到了FANCL蛋白的表达, 说明FANCL在小鼠组织中是广泛表达的, 这与其在DNA修复复合物中的重要成员相一致。

**关键词** FANCL; 抗体; 表达谱

分类号

### Generation of Mouse FANCL Antibody and Analysis of FANCL Protein Expression Profile in Mouse Tissues

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#### Abstract

Fanconi anemia complementation group L (FANCL) is a novel Fanconi anemia protein, which mono-ubiquitinates FANCD2 as a ubiquitin E3 ligase, and plays a crucial role in DNA damage repair and chromosome stability maintenance. FANCL is involved in the proliferation of primordial germ cells (PGC) in early embryonic stages, and may play a role in the development of germ cells by forming a novel testis-specific network with testis-specific proteins in the adult testis. FANCL cDNA sequence was cloned by RT-PCR from mouse testis total RNA, and expressed in *E. coli* BL21 (DE3). Rabbit FANCL polyclonal antiserum was generated using the recombinant protein as the antigen. To prepare an antigen column for affinity purification of FANCL-specific antibody, recombinant His-tagged FANCL was purified by Ni<sup>2+</sup>-charged HiTrap Chelating HP column and coupled to an NHS-activated HiTrap column. To confirm the activity and specificity of the FANCL antibody, we constructed plasmid pCMV-HA/FANCL to transfect HEK 293T cells. Transiently expressed HA-FANCL fusion protein was analyzed by immunoblotting with both the FANCL antibody and HA monoclonal antibody. The antibody was used in Western blotting to check the expression of FANCL protein in mouse tissues. We found wide expression of FANCL in brain, muscle, heart, lung, liver, spleen, kidney, testis, ovary and uterus, indicating the functional importance of this novel protein.

**Key words** FANCL; antibody; expression profile.

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