

Product Datasheet

beta Tubulin Antibody (HRP conjugated)

Catalog No: AB2002

Reactivity: Human Mouse Rat

Isotype: Rabbit IgG

Applications: WB



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Information

UniProt ID: Q13509

All Names: beta-4; CFEOM3A; MC1R; TBB3; TUBB3; TUBB4; Tubulin beta-3 chain; tubulin beta-4; Tubulin beta-4 chain; Tubulin beta-III; tubulin, beta 3; tubulin, beta, 4, beta tubulin;

Form: Liquid

Storage instructions: Store at +4°C short term. Store at -20°C long term. Avoid freeze / thaw cycle.

Storage buffer: pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.

Purity: Affinity-chromatography

Immunogen: A synthesized peptide

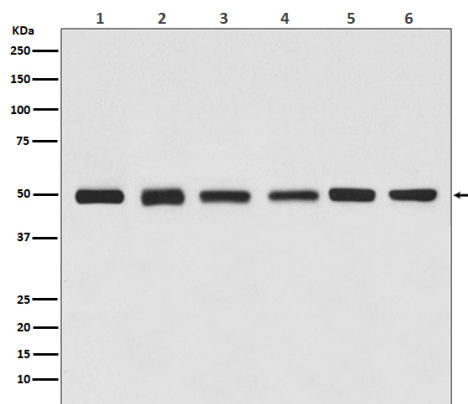
Molecular Wt.: 50 kDa

Application

WB 1:5000~1:20000

Background

Microtubules are constituent parts of the mitotic apparatus, cilia, flagella, and elements of the cytoskeleton. They consist principally of 2 soluble proteins, alpha- and beta-tubulin, each of about 55,000 kDa. Antibodies against beta Tubulin are useful as loading controls for Western Blotting. However it should be noted that levels of beta-Tubulin may not be stable in certain cells. For example, expression of beta-Tubulin in adipose tissue is very low and therefore beta-Tubulin should not be used as loading control for these tissues.



Western blot analysis of beta Tubulin expression in (1) Jurkat cell lysate, (2) Human kidney lysate, (3) 3T3 cell lysate, (4) Mouse brain lysate, (5) C6 cell lysate, (6) Rat heart lysate.

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WB(HRP交联抗体用)操作步骤

操作流程

WB(HRP直标一抗用)操作步骤

1. 常规SDS-PAGE电泳，转印后的PVDF膜，用TBST漂洗掉电转液后，直接放入含有2-5%脱脂奶粉的TBST中（脱脂奶粉要充分溶解在TBST中，不能有奶粉颗粒存在。判断方法：在深色实验台面上，倒少量清水，把脱脂奶粉-TBST溶液加一点到台面的清水中，看是不是溶液状态还是有细小的未溶解奶粉颗粒）。封闭时间：室温1h。封闭时摇床转速不要太快，以液面微微有晃动为准。
2. 封闭结束后，用TBST清洗3次，每次10min。摇床转速要让液面有明显晃动。
3. 第三次TBST清洗后，室温孵育HRP交联后的一抗 1h。一抗需要提前用含有2-5%脱脂奶粉-TBST稀释好。由于一抗已经交联好HRP酶，所以不要过夜孵育一抗。
4. 一抗孵育后，用TBST清洗3次，每次10min。摇床转速要让液面有明显晃动。
5. 清洗后的膜放在曝光板上（HRP交联一抗不需要再孵育二抗），去除多余的TBST液体，但不要膜变干。加上化学发光底物（底物的A液和B液要在室温放置15-30分钟，之后再混合A液和B液）。底物在膜上反应30秒到1分钟后，去掉多余的ECL底物（操作方法：用镊子把膜垂直夹起，膜上ECL底物液体重力自然下流，然后用滤纸把膜下边缘的多余ECL底物吸掉）。多余的ECL底物会一边发生发光反应一边到处流动，造成背景发黑。在膜上盖上玻璃纸后，用纸巾轻轻擦拭玻璃纸，赶走玻璃纸和膜之间的气泡，进入暗室曝光用X胶片曝光。或者直接用仪器曝光。

注：磷酸化抗体可以考虑用1-5% BSA-TBST代替脱脂奶粉-TBST。

TBST配方

1×TBST的终浓度为Tris:0.02mol/L，NaCl:0.1mol/L，吐温-20:0.1%，pH值为7.4。