

## ORDERING INFORMATION

**Catalog Number:** AF4005

**Lot Number:** YKX01

**Size:** 100 µg

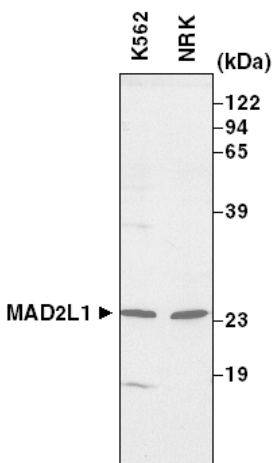
**Storage:** -20° C

**Specificity:** human/rat MAD2L1

**Immunogen:** *E. coli*-derived rhMAD2L1  
(aa 1 - 205)

**Ig Type:** goat IgG

**Application:** Western blot



**Figure 1:** Extracts from exponentially growing K562 and NRK cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/mL goat anti-human MAD2L1 antibody.

## Background

MAD2L1 (Mitotic arrest deficient protein 2) is a component of the spindle-attachment checkpoint mechanism that monitors kinetochore spindle attachment and leads to the subsequent arrest in early metaphase by its recruitment to unattached kinetochores. The transition from metaphase to anaphase requires the association of the anaphase promoting complex/cyclosome (APC/C) with Cdc20 leading to the ubiquitylation and subsequent degradation of Pds1/Securin. This transition is delayed by the inhibitory association of MAD2L1 with Cdc20. MAD2L1 has also been shown to be a direct E2F target and as such is aberrantly expressed in cells with retinoblastoma pathway defects.

## Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human MAD2L1 (rhMAD2L1; aa 1 - 205; GenBank # NM\_002358). Polyclonal antibody was affinity-purified on a column derivatized with rhMAD2L1 and further purified by isolating the IgG fraction.

## Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## Reconstitution

Reconstitute the antibody with 100 µL of sterile PBS containing 0.02% NaN<sub>3</sub>.

## Storage

Lyophilized samples are stable for 12 months from date of receipt when stored at -20° C to -70° C. The reconstituted antibody should be aliquoted and stored at -20° C in a manual defrost freezer for 12 months without detectable loss of activity. **Avoid repeated freeze/thaw cycles.**

## Specificity

The antibody detects human and rat MAD2L1.

## Application

**Western blot** - An antibody concentration of 1.0 µg/mL is recommended. **Optimal dilutions should be determined by the individual laboratory.**

## Protocol for Immunoblotting:

| Blotting Buffer    | Blocking Solution                     |
|--------------------|---------------------------------------|
| 25 mM Tris, pH 7.5 | 5% nonfat dry milk in blotting buffer |
| 0.15 M NaCl        | pH to 7.5                             |
| 0.05% Tween 20     |                                       |

1. Transfer the electrophoresed proteins onto a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2° - 8° C in Blocking Solution containing 1.0 µg/mL goat anti-MAD2L1 antibody.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Blocking Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-goat Ig (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

**Cell lysates for Western blotting:** To prepare total cell lysates, solubilize cells in 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) and sonicate with a probe sonicator using 3 - 4 bursts of 5 - 10 seconds each. Heat extracts in a boiling water bath for 5 minutes and load onto polyacrylamide gels. Samples may be diluted with 1X SDS sample buffer to the desired concentration.