

ANTIBODY TESTING REPORT

SUMMARY

Antigen: Cyclin D1 (Uniprot# P24385)

Method tested: Immunohistochemistry

Laboratory ID: LAB07

Project ID: AR142

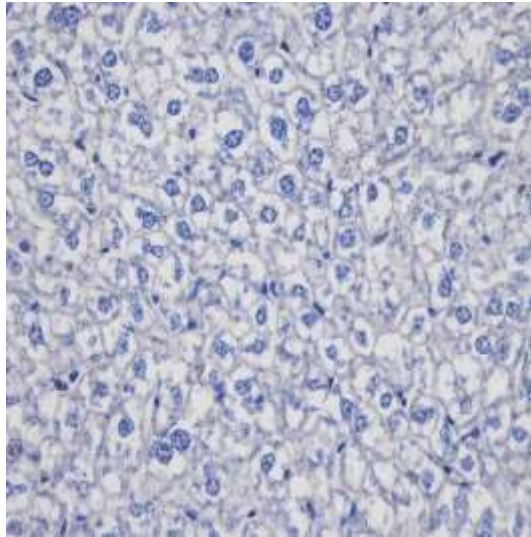
With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical protocol. In doing so, we hope to enable scientists to form an unrivalled opinion of which is the most suitable antibody for their research and in particular, which is going to require the least amount of optimisation, a process which can often take weeks or months.

For the purposes of the antibody comparison initiative, we select the best antibodies from each manufacturer and then compare them side-by-side using the same experimental conditions to provide a direct comparison. The antibodies are collected centrally, repackaged and given an internal reference ID prior to delivery to independent laboratories to ensure objective testing and to minimise bias.

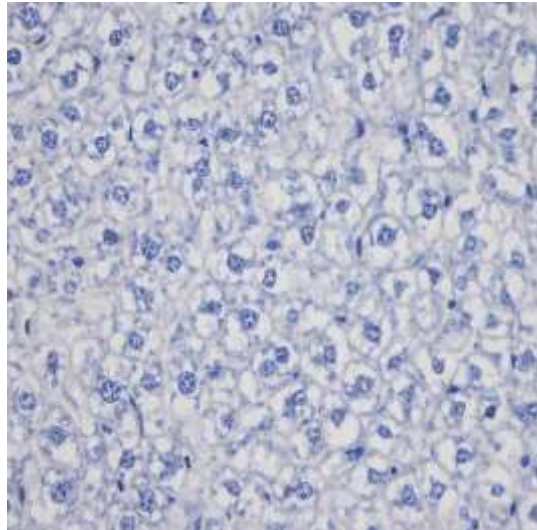
Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS

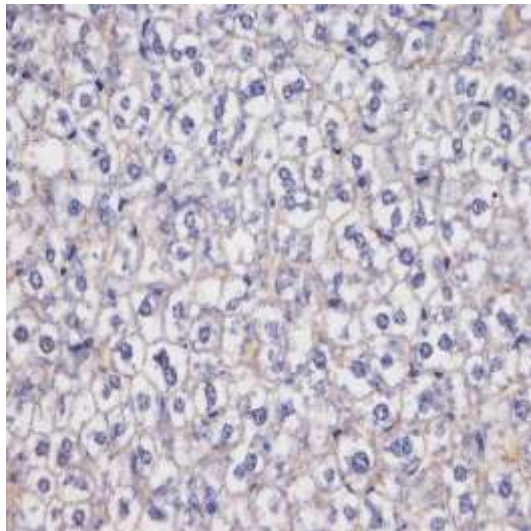
Immunohistochemical analysis of formalin fixed, paraffin embedded Mouse liver tissue using various anti-Cyclin D1 antibodies and isotype controls (see Method section for more detail).



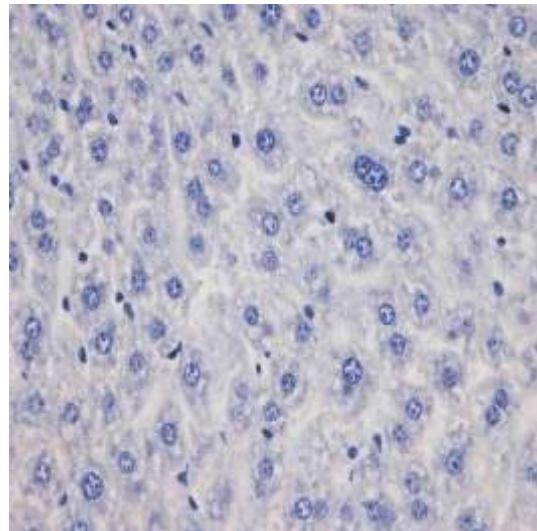
Antibody : Cyclin D1 M121 at 1/50



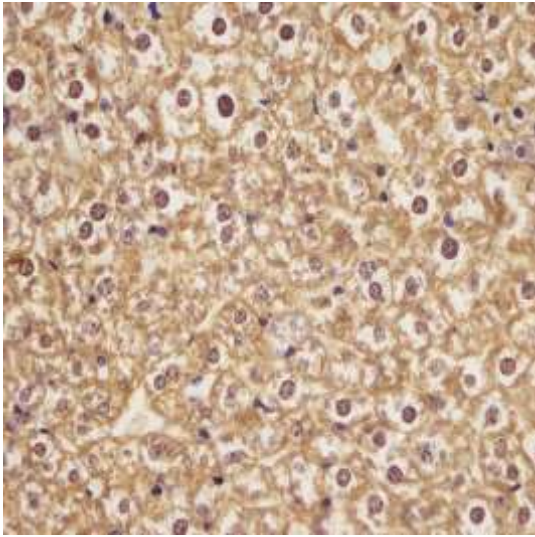
Antibody : Isotype control



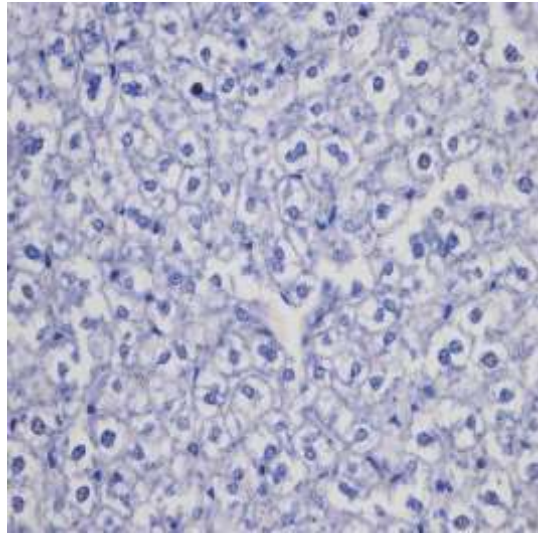
Antibody : Cyclin D1 P102 at 1/50



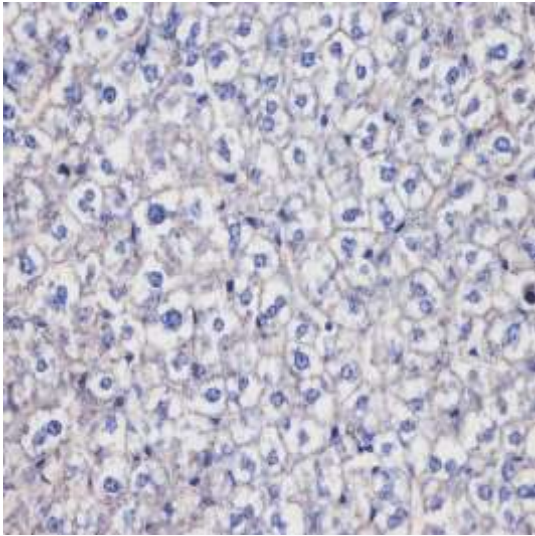
Antibody : Isotype control



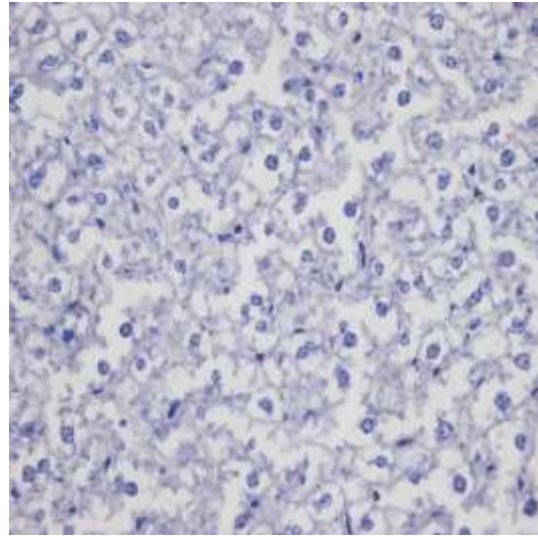
Antibody : Cyclin D1 M153 at 1/50



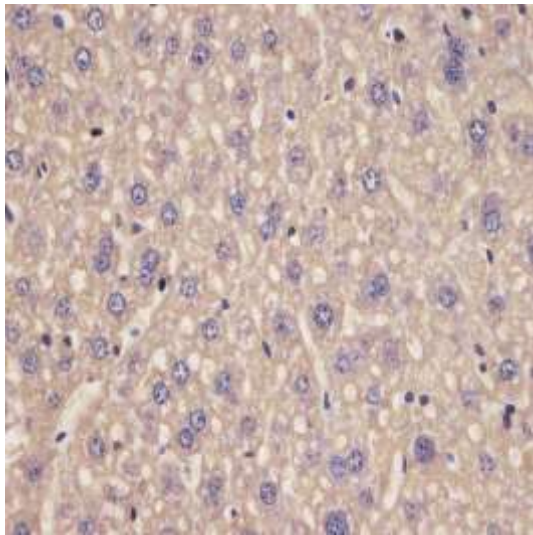
Antibody : Isotype control



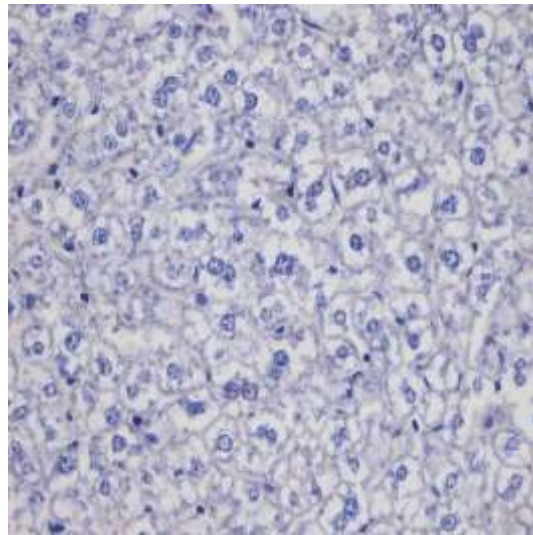
Antibody : Cyclin D1 P148 at 1/50



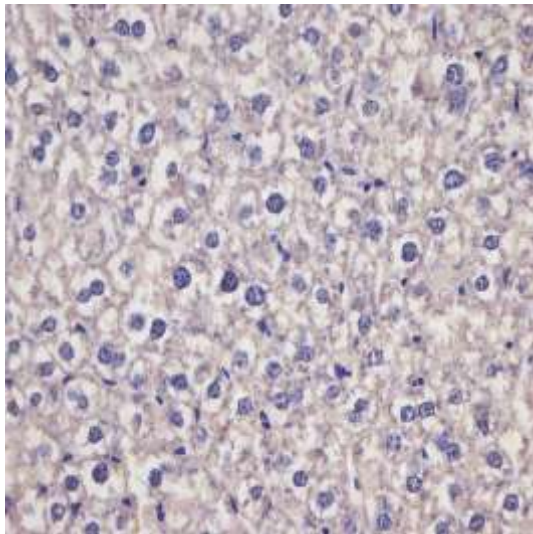
Antibody : Isotype control



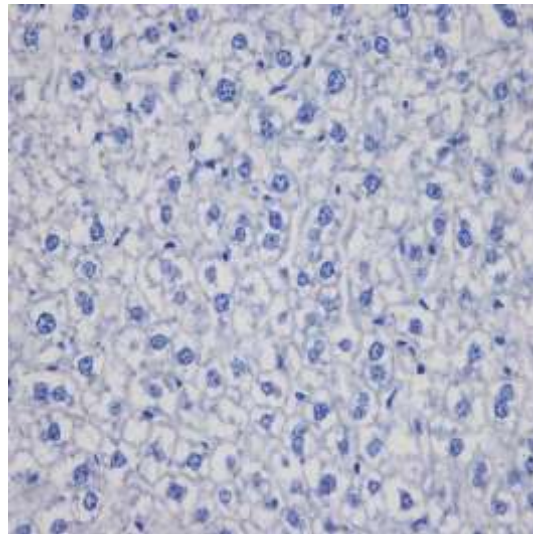
Antibody : Cyclin D1 P160 at 1/50



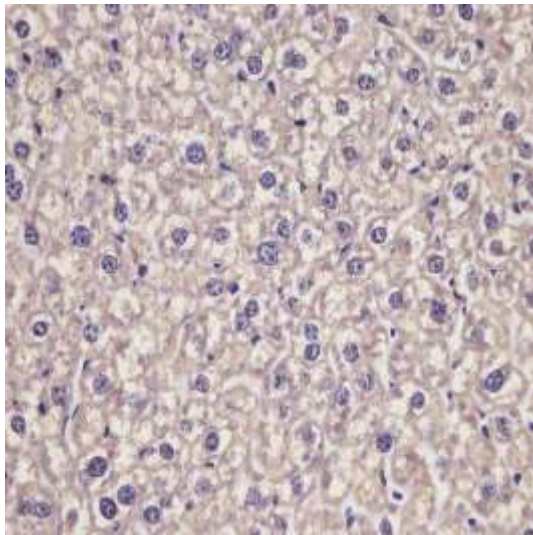
Antibody : Isotype control



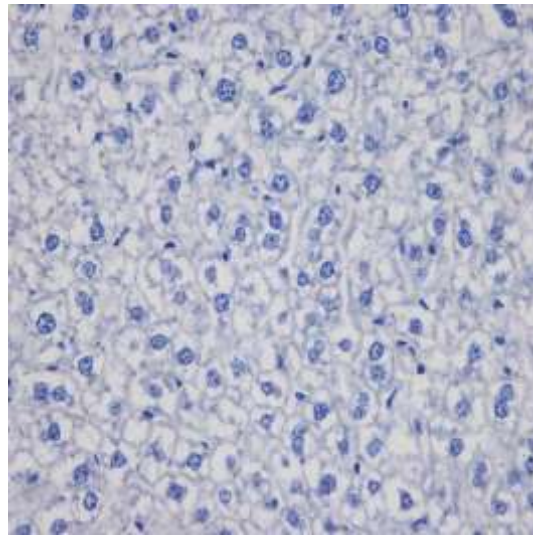
Antibody : Cyclin D1 M191 at 1/50



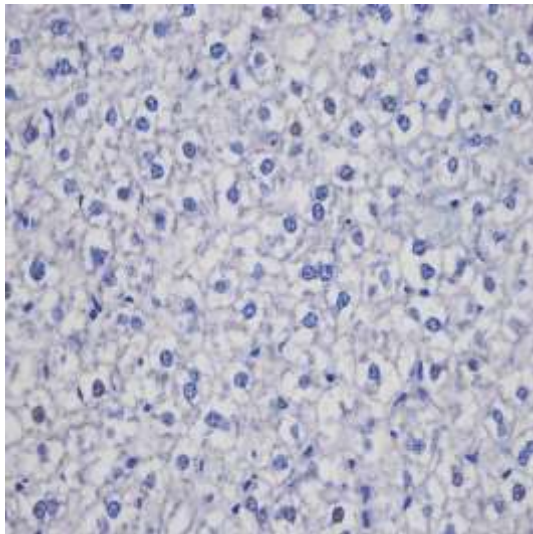
Antibody : Isotype control



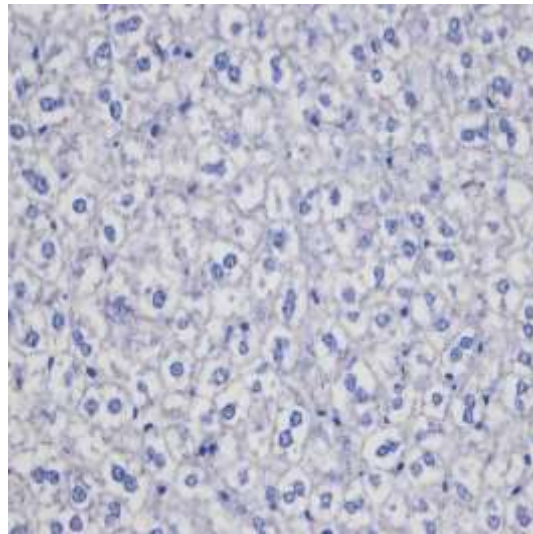
Antibody : Cyclin D1 M194 at 1/50



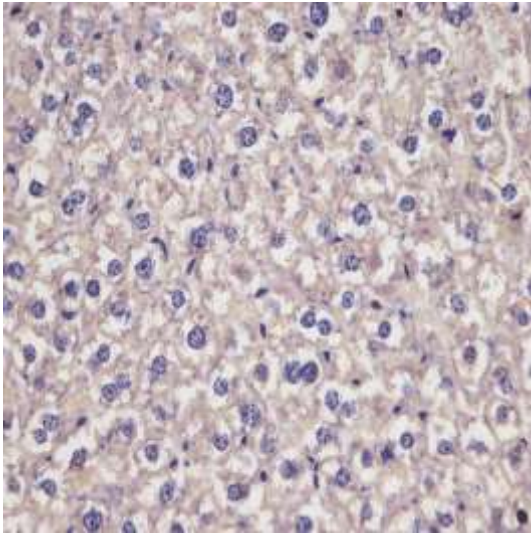
Antibody : Isotype control



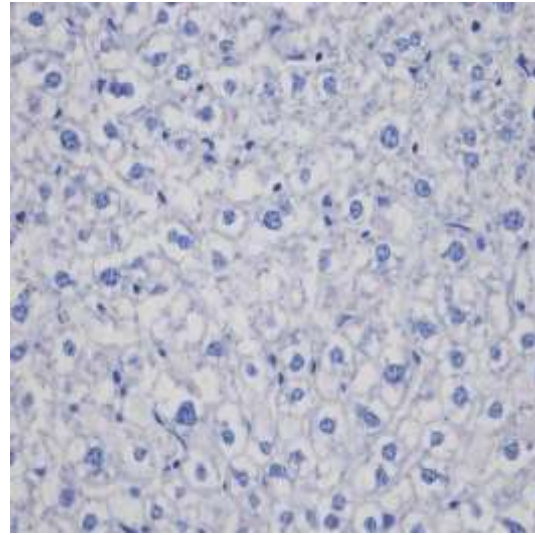
Antibody : Cyclin D1 M196 at 1/100



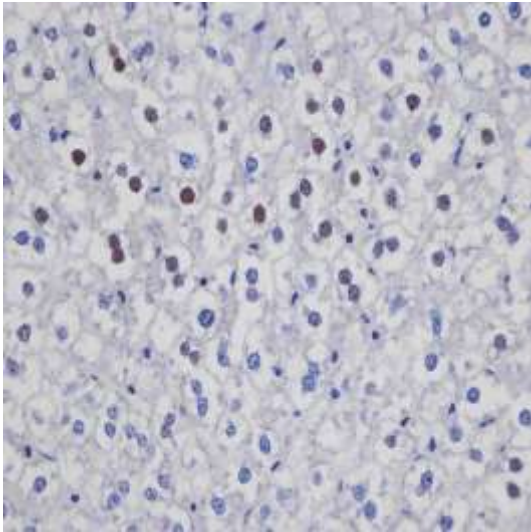
Antibody : Isotype control



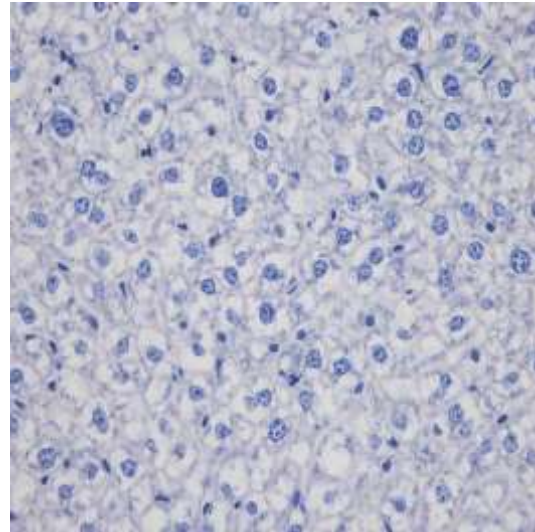
Antibody : Cyclin D1 M198 at 1/50



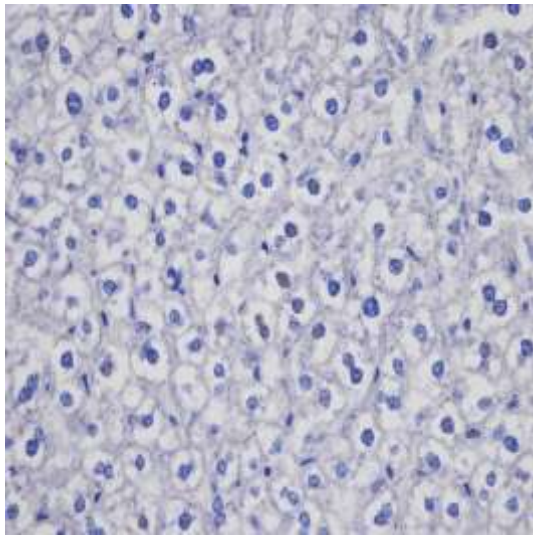
Antibody : Isotype control



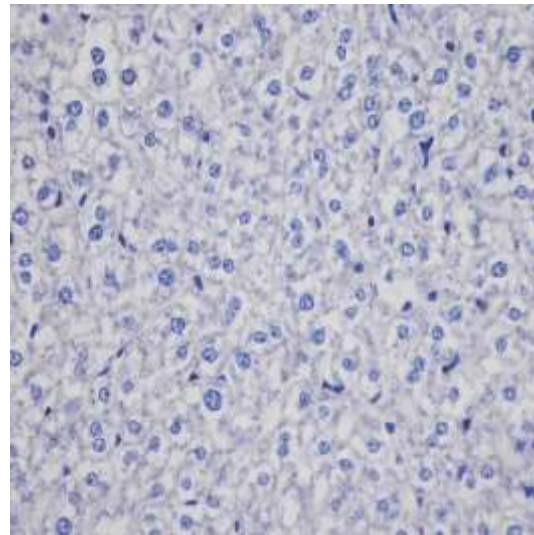
Antibody : Cyclin D1 M200 at 1/50



Antibody : Isotype control



Antibody : Cyclin D1 M202 at 1/100



Antibody : Isotype control

METHOD

Antibodies

Primary antibody	Secondary antibody	Isotype Control
Cyclin D1 M121 at 1/50 (Supplier 13)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/100
Cyclin D1 P102 at 1/50 (Supplier 22)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/100
Cyclin D1 M153 at 1/50 (Novus)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Mouse IgG2a Isotype Control (ThermoFisher Scientific, MA5-14441) at 1/100
Cyclin D1 P148 at 1/50 (Supplier 31)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/100
Cyclin D1 P160 at 1/50 (Supplier 34)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/100
Cyclin D1 M191 at 1/50 (Supplier 19)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Mouse IgG2a Isotype Control (ThermoFisher Scientific, MA5-14441) at 1/50

Cyclin D1 M194 at 1/50 (Supplier 19)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Mouse IgG2a Isotype Control (ThermoFisher Scientific, MA5-14441) at 1/50
Cyclin D1 M196 at 1/100 (Invitrogen)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/100
Cyclin D1 M198 at 1/50 (Supplier 19)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Mouse IgG2a Isotype Control (ThermoFisher Scientific, MA5-14441) at 1/50
Cyclin D1 M200 at 1/50 (Invitrogen)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/50
Cyclin D1 M202 at 1/100 (Invitrogen)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/100



= Component of the Cyclin D1 Superstarter Antibody Panel. See end of report for details.

PROTOCOL

Immunohistochemical analysis of formalin fixed, paraffin embedded Mouse liver tissue was performed using Nikon's DS-Ri1 system.

1. Tissue slides were preheated in convection oven at 60°C for 30min.
2. Deparaffinization was performed by immersing the slides three times in xylene for 10 minutes each time, followed by 5 minutes in 100% ethanol; then 5 minutes in 95% ethanol, 5 minutes in 80% ethanol, 5 minutes in 70% ethanol and finally three washes in distilled water of 5 minutes per wash.
3. An antigen retrieval procedure was then performed by heating the tissue slides, immersed in 10mM sodium citrate buffer, pH 6.0, in a microwave for 8 – 15 minutes. The slices were then allowed to cool at room temperature for 20 – 30 minutes.
4. Endogenous peroxidases were blocked by soaking the slides in 3% hydrogen peroxide-methanol for 15 minutes at room temperature, followed by two washes in distilled water of 5 minutes per wash and one wash of 5 minutes in PBS. Blocking was completed by incubating the slices in 3% BSA in PBS for 30 minutes at room temperature.
5. The slides were then immersed in the primary antibody solution diluted in PBS containing 3% BSA at 37°C for 1 hour or overnight at 4°C in a humidified chamber. Each antibody was diluted according to the working range suggested by the supplier (for details see table above).

6. Following three washes for 5 minutes each wash at room temperature with PBS-Tween (PBST), the slides were incubated in the biotinylated secondary antibody (for details see table above) for 30 minutes at 37°C in a humidified chamber.
7. After removal of the secondary antibody solution, the slides were washed three times for 5 minutes per wash in PBST and then incubated in Streptavidin-HRP solution for 10 minutes at 37°C.
8. DAB staining solution was immediately added and the slides observed until the desired colour change was obtained (typically between 30 seconds and 5 minutes). After draining away excess solution, the slides were placed into distilled water for 5 minutes.
9. The slides were then incubated with haematoxylin for 3 minutes as counterstain.
10. Following three washes with distilled water, the slides were dehydrated by subsequent 5 minute washes in 70% ethanol, 80% ethanol, 95% ethanol, twice with 100% ethanol and two 10 minute washes with xylene. A coverslip was secured on each slide
11. The resulting staining of the tissue was observed and recorded.

EXPERIMENTAL NOTES

The Genecards® entry for Cyclin D1 describes its subcellular localisation as within the nucleus and cytosol. Under these experimental conditions, Cyclin D1 M200 shows nuclear staining. There is also weak nuclear staining in the tissue tested using Cyclin D1 M196 and Cyclin D1 M202, this requires confirmation using more concentrated solutions of these latter two antibodies. Cyclin D1 M153 exhibits nuclear and cytosol staining consistent with the expected subcellular locations of Cyclin D1. Staining is also seen with Cyclin D1 P102, Cyclin D1 P160, Cyclin D1 M191, Cyclin D1 M194 and Cyclin M198 although this seems to be mainly in the cytoplasm whilst no staining is seen using Cyclin D1 M121 or Cyclin D1 P148.

SUPERSTARTER ANTIBODY PANELS



A panel of Superstar antibodies in trial sizes, to enable you to economically test the best antibodies, to determine which is going to be the best for your research project for only \$279, €254, £188.

The Cyclin D1 Superstarter Antibody Panel consists of:

1 x [2978](#) (Cell Signaling Technology)

1 x [sc-8396](#) (Santa Cruz Biotechnology)

1 x [NBP2-24695](#) (Novus Biologicals)

<http://www.antibodyresource.com/superstars>

Images of Superstar Cyclin D1 antibodies:

