**Anti–Dinitrophenyl-KLH Antibodies**

**A-6430** anti-dinitrophenyl-KLH, rabbit IgG fraction  
**A-11097** anti-dinitrophenyl-KLH, rabbit IgG fraction, Alexa Fluor® 488 conjugate  
**A-6435** anti-dinitrophenyl-KLH, rabbit IgG fraction, biotin-XX conjugate  
**A-6423** anti-dinitrophenyl-KLH, rabbit IgG fraction, fluorescein conjugate

### Introduction

Because of their high affinity for the dinitrophenyl hapten\(^1,2\) (DNP), Molecular Probes’ antibodies against the dinitrophenyl–keyhole limpet hemocyanin conjugate (anti–DNP-KLH) are sensitive probes for detecting DNP-labeled molecules\(^3,6\). The fluorescent and biotinylated anti–DNP-KLH conjugates can serve as second-step reagents for signal amplification in protein and nucleic acid detection systems, including *in situ* hybridization studies employing DNP-modified DNA probes.

For labeling nucleic acids and proteins with DNP, Molecular Probes offers DNP-X succinimidyl ester (D-2248) and the FluoroReporter® Biotin/DNP Protein Labeling Kit (F-6348). For detecting biotinylated anti–DNP-KLH in enzyme-linked hybridization and immunoassays using avidin or streptavidin bridge techniques, we prepare a biotin conjugate of horseradish peroxidase (P-917).

For assays not requiring a fluorescent conjugate of the anti-DNP antibody, Molecular Probes offers unconjugated anti–DNP-KLH antibody (A-6430). This antibody can be used for Fc receptor–mediated delivery of DNP-labeled liposomes prepared from DNP phospholipids\(^7\). Because anti-DNP antibody binds well to DAMP (D-1552), an acidotropic reagent that can penetrate cell membranes, anti–DNP-KLH antibodies can also be used with DAMP for immunochemical localization of intracellular compartments with low pH\(^8,11\). In addition, anti–DNP-KLH antibodies may recognize compounds with a trinitrophenyl group, allowing the isolation of cell-surface molecules of human melanoma cells that have been labeled with trinitrobenzene sulfonic acid (TNBS)\(^12\).

### Contents and Storage

The anti–DNP-KLH antibodies are supplied in unit sizes of 0.5 mL as a 2 mg/mL solution. The conjugates (A-6423, A-6435 and A-11097) are in 0.1 M sodium phosphate, 0.1 M sodium chloride, pH 7.5, containing 5 mM sodium azide. The unlabeled antibody (A-6430) is in phosphate-buffered saline (PBS), pH 7.2, containing 5 mM sodium azide. For the conjugates, the degree of labeling is indicated on the product label. The anti–DNP-KLH antibodies are stable for two to three months when stored undiluted at 4°C. For longer storage, divide the solution into aliquots and freeze at -20°C. These aliquots should be stable for at least one year when stored frozen. PROTECT FLUORESCENT CONJUGATES FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

### Properties

Molecular Probes’ anti–DNP-KLH antibody was raised in rabbit against DNP conjugated to keyhole limpet hemocyanin. The IgG fraction, which contains both anti-DNP and anti-KLH IgGs, was isolated from total serum and determined to be >95% pure by SDS-polyacrylamide gel electrophoresis.

It is a good practice to centrifuge protein conjugate solutions briefly in a microcentrifuge before use; only the supernatant should then be added to the experiment. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining.

Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically\(^13\). Peak absorption and fluorescence emission wavelengths for both the fluorescein anti–DNP-KLH and Alexa Fluor® 488 anti–DNP-KLH conjugates are 494 nm and 520 nm, respectively.

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**Storage upon receipt:**  
- 4°C or -20°C (in aliquots)  
- Avoid freeze-thaw cycles  
- Protect fluorescent conjugates from light

**Abs/Em of A-6423 and A-11097:** 494/520 nm
## Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product Name</th>
<th>Unit Size</th>
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</thead>
<tbody>
<tr>
<td>A-6430</td>
<td>anti-dinitrophenyl-KLH, rabbit IgG fraction <em>2 mg/mL</em></td>
<td>0.5 mL</td>
</tr>
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<tr>
<td>A-6423</td>
<td>anti-dinitrophenyl-KLH, rabbit IgG fraction, fluorescein conjugate <em>2 mg/mL</em></td>
<td>0.5 mL</td>
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</tbody>
</table>

## References


## Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — [www.probes.com](http://www.probes.com) — for the most up-to-date information.

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