TALKS: FLUORESCENT LABELLING

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Genetics and Molecular Biology
TALKS: FROM BENCH TO PUBLICATION

LABELING
AUG 13 HSRB  AUG 16 WBRB

TECHNIQUE AWARENESS
SEPT 10 WINSHIP  SEPT 13 WBRB

ACQUISITION
SEPT 24 HSRB  SEPT 27 WBRB

OPTIMIZATION

DATA ANALYSIS
OCT 8 WINSHIP  OCT 11 WBRB
WHY FLUORESCENCE MICROSCOPY?
What I’d like our users to know

WHAT IS FLUORESCENCE? (5%)
IMMUNOFLUORESCENCE OPTIMIZATION (55%)
FLUORESCENT PROTEINS (20%)
LIVE CELL DYES (5%)
NEW TECH (10%)
WHAT IS FLUORESCENCE?
Stokes Shift

Energetic shorter wavelengths are absorbed, longer wavelengths with less energy are emitted.
Excitation and Emission

Graph showing the % Normalized Excitation/Emission peaks for different spectral peaks:
- Dapi at 405 nm
- GFP at 486 nm
- Alexa 594 at 513 nm
- Cy5 at 633 nm

Wavelength (nm) range: 300 to 700 nm
Brightness/
Quantum Yield

Photons emitted
Photons absorbed
What I’d like our users to know

IMMUNOFLUORESCENCE OPTIMIZATION (55%)
FLUORESCENT PROTEINS (20%)
LIVE CELL DYES (5%)
NEW TECH (10%)
Immuno-fluorescence
WHAT IS AN ANTIBODY?

Monoclonal antibody, represents antibody from a single antibody producing B cell and therefore only binds with one unique epitope.
Fixation ▼
Permeabilization ▼
Primary Antibody

<table>
<thead>
<tr>
<th>Constant region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable region</td>
</tr>
<tr>
<td>Antigen (Polypeptide)</td>
</tr>
</tbody>
</table>
Rabbit conserved region

Product Information

Anti-Actin produced in rabbit, affinity isolated antibody

Catalog Number: A2066

Product Description
Anti-Actin is produced in rabbit using C-terminal actin fragment (C11 peptide attached to Multiple Antigen Peptide (MAP) backbone) as immunogen. The sequence is Ser-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-Cys-Pro-Pheny. Affinity isolated antibody is obtained from rabbit anti-actin antiserum by immunoprecipitation purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to actin.

Anti-Actin specifically stains typical stress fibers in cultured chicken fibroblasts using indirect immunofluorescent labeling and specifically localizes actin by immunoperoxidase labeling of formalin-fixed, paraffin-embedded human or animal tissue sections following enzymatic unmasking. In immunofluorescence, the product localizes actin in many species ranging from human skeletal muscle to amoeba. The product recognizes the 42 kDa actin band using immunoblotting with human or animal tissue extracts.

The antibody is useful for studying actin structure and function, and to probe binding sites of actin-binding proteins.

Actin, a highly conserved protein, is a major component of both the cytoskeletal and contractile structures in all cell types. It varies in amount, being related to the type of differentiation and to the functional state of cells and tissues. Actin can be found in two different forms of aggregation, the globular or the fibrillar state, and at least six distinct isoforms occur in vertebrates. The actins exhibit over 95% sequence homology, but each isoform has a unique N-terminal sequence. The isoforms are comprised of three a actins (skeletal, cardiac, smooth), one b actin (b- non-muscle) and two y actins (y smooth muscle and y non-muscle). Difficulties have been encountered in producing polyclonal antibodies due to the highly conserved nature of actin. Because the amino acid sequence of the C-terminal region is the same for almost all actins,

this antibody has been raised using a synthetic peptide corresponding to the C-terminal 11 residues.

Reagents
Supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as preservative.

Protein Concentration: 0.4-0.8 mg/ml by absorbance using E 1% = 14.0 (prior to the addition of BSA),

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2 & 8 °C for up to one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

Immunohistochemistry: a working dilution of at least 1:20 was determined using formalin-fixed paraffin embedded human or animal tissues.

Immunofluorescence: a working dilution of at least 1:100 was determined using chicken gizzard extract.

Note: In order to obtain best results, it is recommended that each individual user determine their optimum working dilution by titration assay.

IF concentration 1:40

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Optimize your antibody

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Fixation
Formaldehyde, gluteraldehyde, alcohol, acetone

Permeabilization
Triton, tween, saponin, alcohol, acetone

Concentration and Block

Antigen (Polypeptide)
Optimize your antibody

Positive and Negative Controls are necessary

Antigen (Polypeptide)
Optimize separately than together

Antigen (Polypeptide)

Variable region

Constant region
Secondary Antibody
Donkey conserved region

Labeled Donkey Anti-Mouse IgG Antibodies

Table 1. Content and storage information.

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescein-labeled donkey anti-mouse IgG</td>
<td>0.5 ml 2 mg/ml</td>
<td>−20°C -8°C</td>
<td>When stored undiluted as</td>
</tr>
<tr>
<td>(H1L1) antibody</td>
<td>solution in 0.1</td>
<td>protect from light</td>
<td>directed, antibodies are</td>
</tr>
<tr>
<td>sodium phosphate, 0.1 M NaCl, pH 7.3, 3 m</td>
<td>mL sodium</td>
<td>stable for at</td>
<td></td>
</tr>
<tr>
<td>salt</td>
<td>phosphate,</td>
<td>least 3 months.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 7.3, 3 m</td>
<td>* For longer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sodium</td>
<td>storage, dilute</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phosphate,</td>
<td>the solution</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 7.3, 3 m</td>
<td>into aliquots</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sodium</td>
<td>and freeze at</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phosphate,</td>
<td>−20°C. Frozen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 7.3, 3 m</td>
<td>aliquots are</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sodium</td>
<td>stable for at</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phosphate,</td>
<td>least 6 months.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 7.3, 3 m</td>
<td>* Avoid repeated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sodium</td>
<td>Freezing and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phosphate,</td>
<td>Thawing.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 7.3, 3 m</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Degree of labeling: The degree of labeling for each conjugate is typically >98% fluorescein molecules per IgG molecule; use the degree of labeling indicated on the product label.

Approximate fluorescence excitation/emission maxima: See Table 2.

Introduction

Molecular Probes fluorescent donkey anti-mouse IgG antibodies are prepared from affinity-purified antibodies that react with IgG heavy chains and all classes of immunoglobulin light chains from mouse. The Alexa Fluor<sup>®</sup> dyes to which these antibodies are conjugated provide for extraordinarily bright antibody conjugates. The donkey anti-mouse IgG antibodies show minimum cross-reactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, and sheep serum proteins. The approximate excitation and fluorescence emission maxima for each of the conjugates are shown in Table 2.

In addition to the secondary antibodies described in this manual, Molecular Probes prepares fluorescent conjugates of many other species-specific anti-IgG antibodies, as well as conjugates of avidin, streptavidin, NeutrAvidin<sup>™</sup> biotin-binding protein, protein A, and protein G. Please consult our website at prebs.invitrogen.com or contact our Technical Service Department for more information about these products.

At the time of preparation, the products are certified to be free of un conjugated dyes and are tested in a cytological experiment to ensure low nonspecific staining.

Table 2. Labeled donkey anti-mouse IgG antibodies.*

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Label</th>
<th>Ex (λ)</th>
<th>Em (λ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A21202</td>
<td>AlexaFluor&lt;sup&gt;®&lt;/sup&gt; 647</td>
<td>630</td>
<td>668</td>
</tr>
<tr>
<td>A21203</td>
<td>AlexaFluor&lt;sup&gt;®&lt;/sup&gt; 594</td>
<td>590</td>
<td>617</td>
</tr>
<tr>
<td>A21214</td>
<td>AlexaFluor&lt;sup&gt;®&lt;/sup&gt; 488</td>
<td>490</td>
<td>519</td>
</tr>
<tr>
<td>A21216</td>
<td>AlexaFluor&lt;sup&gt;®&lt;/sup&gt; 555</td>
<td>555</td>
<td>583</td>
</tr>
</tbody>
</table>

*Fluorescent conjugates to bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, and sheep serum proteins. Approximate excitation and fluorescence emission maxima at which antibodies can be excited.

Storage

Know your spectrum

Excitation and Emission
Consider mixing VERY carefully

Rabbit anti-mouse 488

Donkey anti-Rabbit 568
Consider mixing VERY carefully

Rabbit anti-mouse 488

Donkey anti-Rabbit 568

Co-localized?
Preferred: same animal, same block

- Donkey anti-mouse 488
- Donkey anti-Rabbit 488
Know your spectrum

Fluorophore Selection

Light Sources

Important Notes:
OMX Sample Prep Recommendations
Mount with non-hardening mounting media

Non-hardening varieties (Vectashield) help preserve 3D structure
SMALLER ANTIBODIES
DO YOU HAVE ANY IF QUESTIONS?
What I’d like our users to know

FLUORESCENT PROTEINS (20%)
LIVE CELL DYES (5%)
NEW TECH (10%)
Green Fluorescent Protein

Stable expression
DNA Expression:
When is it expressed?
How much?
In which cells?
Endogenous loci?

Protein Expression:
N-terminal vs C
Confirmation (folding)
 Trafficking
Proper localization
How large is the FP
DNA Expression:
When is it expressed?
How much?
In which cells?
Endogenous loci?

Protein Expression:
N-terminal vs C-terminal
Confirmation (folding)
Trafficking
Proper localization
How large is the FP

Positive and Negative Controls are necessary
Alternative Splicing

DNA

RNA

mRNA

Translation

Protein A

Protein B

Protein C
BRAINBOW: Cre-Lox recombination of DNA with Fluorescent proteins
Ratiometric Fluorescent Indicators
DO YOU HAVE ANY FLUORESCENT PROTEIN OR GENETICS QUESTIONS?
CONSULTATION: THINGS TO CONSIDER BEFORE YOU COME TO A CONFOCAL

- Fluorophores match etc.
- Positive and negative controls, antibody dilution
- Check your slides on a fluorescent widefield (you need another set of eyes?)
- Navigation markers
- What would you like as a final figure (how you should shoot and analyze)
What I’d like our users to know

- LIVE CELL DYES (5%)
- NEW TECH (10%)
### Live Cell Dyes

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live/fixed (cells or tissue)</td>
<td></td>
</tr>
<tr>
<td>Live/fixed (aldehyde)</td>
<td></td>
</tr>
<tr>
<td>Live/fixed (aldehyde)</td>
<td></td>
</tr>
<tr>
<td>Fixed (aldehyde)</td>
<td></td>
</tr>
<tr>
<td>Live (can be fixed in aldehyde after staining)</td>
<td></td>
</tr>
<tr>
<td>Live (can be fixed in aldehyde after staining)</td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td></td>
</tr>
</tbody>
</table>
SNAP AND CLIP

1) sub-cloning and expression of the protein of interest as a CLIP-tag fusion.

Clip tag: 20 kDa mutant of the DNA repair protein O⁶-alkylguanine-DNA alkyltransferase

2) Transfect CLIP-tag fusion into cell

3) Add substrate: benzylguanine (BG) derivatives + Fluorophore
FLUORESCENCE IN SITU HYBRIDIZATION (FISH)
CAN I MAKE A SCIENTIFIC CONNECTION FOR YOU?
Integrated Cellular Imaging
Directors

Adam Marcus, PhD

Neil Anthony, PhD
Image Analyses

William Giang