

RetroBeads™

The original microspheres for retrograde tracing

Product Description

Product name	cat.number	$\lambda_{exc} \backslash \lambda_{em}$ max. (nm)
Red RetroBeads™	FP-DX2090, 100 µl	530 / 590
Red RetroBeads™ IX	FP-DX2100, 100 µl	530 / 590
Green RetroBeads™ IX	FP-DX2110, 100 µl	460 / 505

Storage: The bead solution should be stored in a humidified container, in a refrigerator, to prevent evaporation. **Do not freeze!** Beads that have been frozen will not work, and cannot be rescued. **If the beads dry out, they cannot be reconstituted.** No shelf life has been established for this material, but, when properly stored, it remains good for several years.

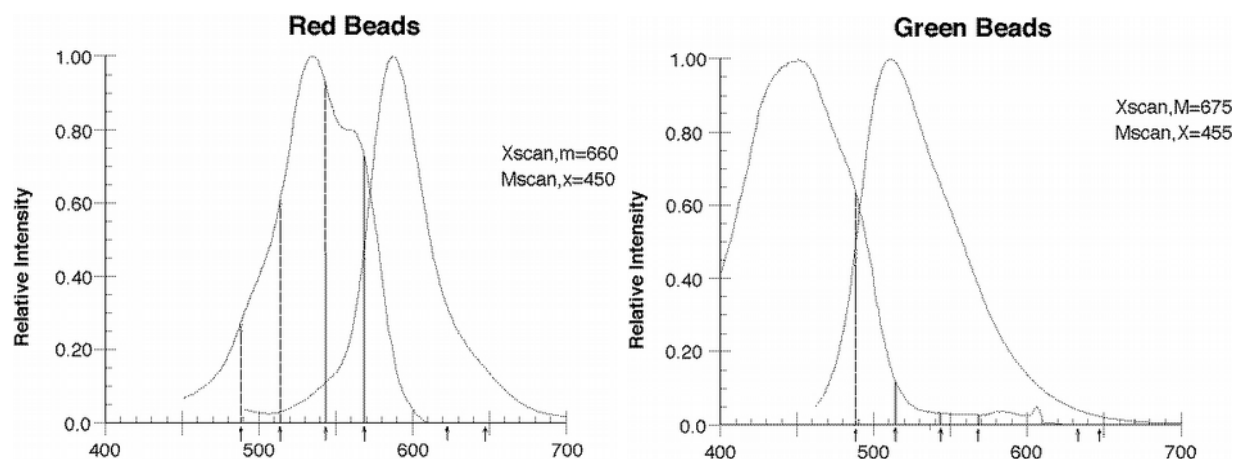
Introduction

- Highly confined injections: superb for detailed connectivity studies.
- Persist indefinitely (> 1 year!) in living cells, nontoxic.
- Compatible with most other anterograde tracers, *in situ* hybridization, and immunohistochemistry.

Retrobeads™ IX: Retrobeads™ deliver bioactive agents (such as neurotrophins and neurotransmitter agonists/antagonists) to localized regions; retrograde transport allows determining which neurons were exposed to the agents [Riddle et al. Nature 378:189, 1995 and Quattrocchi et al. Science 245:984, 1989].

Retrobeads™ IX are specially prepared to facilitate adsorption of proteins and other bioactive compounds.

Retrobeads™ IX are also more effective tracers in primate systems than standard Retrobeads™.



Directions for use

The enclosed vial(s) contains a concentrated solution of beads suspended in distilled water. If red beads are being used for retrograde tracing of neuronal pathways, the solution can be used as is, or diluted. In rat visual cortex, dilutions of 1:4 do not appear to reduce the quality or extent of retrograde labeling when using red beads. However, for initial experiments we strongly recommend using the solution full strength. In addition to distilled water, standard salt solutions (NaCl, KCl) can be used as diluents. The green beads, as supplied, are completely prepared for retrograde tracing experiments. **Dilution of green beads is not recommended.**

Guidelines for use

Beads are best injected using pressure (e.g. a 1 ml Hamilton syringe, or pressurized air injection system). For local circuit work, very small volumes (30-50 nl) have been injected through glass pipettes with 30-50 mm diameter tips. For routine retrograde tracing, larger volumes (0.1-0.3 ml) and larger diameter pipette tips are used. However, even with large injections beads do not diffuse far from the injection site (usually less than 1 mm). Thus in order to label all or most of the neurons projecting to a large structure, several injections should be made. Iontophoretic application of beads is not recommended as an effective means to deliver the tracer. However, the beads do have a net negative charge.

Survival times: The minimum effective post-injection survival time in most warm-blooded vertebrate systems is approximately 24 hours. Labeling intensity increases with longer survival, up to 48 hours. After 48 hours, no increase (or decrease) in labeling intensity is observed. These values may be considerably different in cold-blooded animals, and initial survival times of a week are recommended. The maximum survival time has not been established, but labeling is unchanged in either quality or extent even after 14 month survival times. Cells probably are permanently marked. No toxic effects on either animals or neurons have been observed.

Fixation and processing: Standard fixation is a 0.1 M phosphate buffer wash followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Glutaraldehyde will produce substantial tissue autofluorescence which may obscure bead-labeled neurons, and should be avoided if possible. Green beads will be completely invisible in glutaraldehyde fixed material. Frozen sections are collected in phosphate buffer, mounted on gelatin-coated slides, and air-dried. After complete drying, slides can be cleared in xylene for 1 minute, and covers lipped with Fluoromount or Krystalon. Brief exposures to alcohols and xylenes are not harmful, but long exposures (over 5 minutes) will destroy the beads. Beads are very sensitive to glycerol, and will fade rapidly if mounted in glycerol-containing solutions. Methyl salicylate is preferable to glycerol in situations where non-permanent clearing/mounting agents are indicated. If slides are kept in the dark, the labelling in cells will not fade for at least one year (although background autofluorescence may increase substantially). Thus far, attempts to retain bead labeling after plastic embedding have not been successful.

Observation: The dye in the red beads is rhodamine, thus any fluorescence filter set for rhodamine can be used. Some older Nikon rhodamine filter sets give a very high background, which can make labeled cells invisible. Good results have been obtained with both Zeiss and Leitz standard rhodamine filters. For green beads, a wide-band fluorescein filter works well. Filter sets for Lucifer yellow give a more intense signal, but at the expense of higher background. Narrow-band fluorescein filters will give a much weaker signal than a broad-band filter. Beads do not fade appreciably even after long periods of observation or photomicrography.

Labeling is usually not visible with low power, low numerical aperture dry objectives (e.g. X4, X10). If cells are strongly labeled, a X10 immersion objective (numerical aperture of 0.4 or greater), or higher power dry objectives, will usually reveal the cells. However, frequently a X25 immersion objective will reveal very clearly labeled cells that lower power objectives miss. These caveats are especially true for green beads. Before deciding that an experiment did not work, examine sections in the vicinity of the injection site with immersion objectives. Numerous locally labeled cells should be present.

Additional information for green bead users: In work that has been done so far, it appears that younger animals transport the label better than older animals. In addition, tissue autofluorescence is lower in the younger animals. Therefore, it is advisable to use younger animals, if possible, in experiments involving green beads.



FT-DX2090

Because the green beads are excited at shorter wavelengths than red beads, tissue autofluorescence is a greater problem. Therefore, efforts to minimize autofluorescence will produce a better contrast signal. Ways to reduce autofluorescence include: 1) using thinner sections (e.g. 30 um rather than 40 or 50) 2) using younger animals, and 3) examining sections promptly after coverslipping (background increases over time).

References

- **Cham J. & Badoer E.**, Exposure to a hot environment can activate rostral ventrolateral medulla-projecting neurones in the hypothalamic paraventricular nucleus in conscious rats, *Exp Physiol*, 93: 64 - 74 (2008) [Article](#)
- **Dugas J. et al.**, A Novel Purification Method for CNS Projection Neurons Leads to the Identification of Brain Vascular Cells as a Source of Trophic Support for Corticospinal Motor Neurons, *J. Neurosci.*, 28: 8294 - 8305 (2008) (RetroBeads IX) [Abstract](#)
- **Katz, L.C., et al.** Fluorescent latex microspheres as a retrograde neuronal marker for in vivo and in vitro studies of visual cortex. *Nature* 310: 498-500 (1984)
- **Katz, L.C. and Iarovici, D.M.** Green fluorescent latex microspheres: a new retrograde tracer. *Neuroscience* 34: 511-520 (1990)
- **Low L. et al.**, Plexin signaling selectively regulates the stereotyped pruning of corticospinal axons from visual cortex, *PNAS*, 105: 8136 - 8141 (2008) [Article](#)
- **Quattrochi et al.** *Science* 245:984, 1989 (RetroBeads IX)
- **Riddle et al.** NT-4-mediated rescue of lateral geniculate neurons from effects of monocular deprivation, *Nature* 378:189, 1995 (RetroBeads IX) [Abstract](#)

Technical and scientific information

Related / associated products and documents

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- Fluoromount, [FP-483331](#)
- Hydroxystilbamidine (FluoroGold), [FP-40766A](#)
- Hydroxystilbamidine 4% Solution (FluoroGold), [FP-JW7290](#)
- Fast Blue, neuronal tracer, [FP-CM0241](#)

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